8C-0895-13545

PPSC

PETROLEUM PRODUCT STEWARDSHIP COUNCIL

1100 NEW YORK AVENUE, N.W., SUITE 1090, WASHINGTON, D.C. 20005 • (202) 414-4100 • FAX (202) 289-8584

Contains No CBI

August 5, 1996

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RETURN RECEIPT REQUESTED ON REVISED LETTER

Docket Processing Center
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
RM G - 099
401 M Street, SW
Washington, D.C. 20460

ATTN: TSCA Section 8(e) Health and Safety Reporting Rule

COMPOUND: Naphtha (petroleum), light alkylate, CAS No. 64741 - 66 - 8

RE: Revised cover letter

Dear Sir or Madam,

Enclosed please find a revised cover letter for the LAN aquatic toxicity TSCA 8(e) final report filing. PPSC requests that this replace the letter filed earlier today, August 5, 1996.

Thank you,

Paula Podhasky for:

Charles R. Clark, Ph.D., D.A.B.T.
Chairman, Petroleum Product Stewardship Council

8EHQ-95-13545

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Enclosures (5)

89960000194

PPSC

PETROLEUM PRODUCT STEWARDSHIP COUNCIL

1100 NEW YORK AVENUE, N.W., SUITE 1090, WASHINGTON, D.C. 20005 • (202) 414-4100 • FAX (202) 289-8584

August 5, 1996

RETURN RECEIPT REQUESTED

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COMPOUND: Naphtha (petroleum), light alkylate, CAS No. 64741 - 66 - 8

RE: Final Reports for Acute Aquatic Toxicity Studies

Dear Sir or Madam,

On November 6, 1995 I wrote to advise EPA of preliminary aquatic toxicity testing results of light alkylate naphtha (LAN). Enclosed are the final reports on the water accommodated fraction of LAN (Light Alkylate Naphtha). These studies were conducted in closed systems to minimize the loss of volatile hydrocarbons. The Notice of Substantial Risk was received by OPPT CBIC on November 7, 1995. The five report titles are as follows:

- Static-Renewal 48-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to *Daphnia magna*
- Static 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to a Freshwater Alga, *Selenastrum capricornutum*
- Static-Renewal 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to Mysid Shrimp
- Static-Renewal 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to Fathead Minnow
- Static-Renewal 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to Silverside Minnow

This report is being submitted by the Petroleum Product Stewardship Council on behalf of the study sponsors which include ARCO, Amoco Corporation, *BP* America, Inc., Chevron Environmental Health Center, Mobil Oil Corporation, Unocal Corporation and Texaco, Inc.

If there are any questions regarding this submission, please feel free to contact Paula Podhasky at 202 / 414 - 4156.

These documents contain no confidential business information.

Sincerely,

Charles R. Clark (pp)

Charles R. Clark, Ph.D., D.A.B.T.
Chairman, Petroleum Product Stewardship Council

Enclosures (5)

Stonybrook
Laboratories Inc.

Static-Renewal 48-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to *Daphnia magna*

Stonybrook Laboratories Inc.
Princeton, NJ

Final Report

Study Number 65907

STONYBROOK LABORATORIES INC.
REPORT RELEASE

TO STUDY DIRECTOR/LIAISON: C.A. Schreiner

STUDY NUMBER: 65907

CRU NUMBER: 94124

SAMPLE NAME: Whole Light Alkylate Product

STUDY TITLE: Static-Renewal 48-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to *Daphnia magna*

REQUESTER: Petroleum Product Stewardship Council

RESULTS:

EC_{50} 32 ppm for Whole Light Alkylate Product (Nominal)
EC_{50} 556 ppb for Whole Light Alkylate Product (Measured)

A static-renewal 48-hour toxicity study was conducted November 21-23, 1994 to determine the acute toxicity of Whole Light Alkylate Product to *Daphnia magna*, a representative freshwater invertebrate species. Test daphnids were exposed to individual water accommodated fractions (WAF) of the poorly water-soluble test material at nominal concentrations of 9 ppm, 18 ppm, 35 ppm, 70 ppm, and 140 ppm (w/v, based on density). Nominal test concentrations are based on the loading rate, or amount of test material added to make each WAF. Test solutions were renewed daily during the study. Water quality parameters of pH, temperature, dissolved oxygen (D.O.), conductivity, alkalinity, and hardness were measured for the test chambers throughout the study.

Samples of the control and exposure concentrations were collected at 0, 24, and 48 hours and quantitatively analyzed using purge-and-trap/gas chromatography (GC). The concentrations were quantitated using standard Whole Light Alkylate Product component standards. Measured test concentrations are based on the total concentration of all analytes. Test material retention from the static-renewal procedure ranged from 48.3-66.0%, producing consistent exposure of the test Daphnia to Whole Light Alkylate Product throughout the study.

The toxicity of the test material was evaluated on the basis of EC_{50} determinations at 24 and 48 hours. The term EC_{50} used in this report refers to the concentration immobilizing 50% of the test population after a specified exposure period. The computer-estimated 48-hour EC_{50} for Whole Light Alkylate Product was 32 ppm, based on nominal concentrations, and 556 ppb, based on measured concentrations. The 48-hour no observed effect concentration (NOEC), based on nominal concentrations, was 18 ppm, since exposure to concentrations of 35 ppm and greater resulted in significant immobility. The 48-hour no observed effect concentration (NOEC), based on measured concentrations, was 339 ppb, since exposure to concentrations of 596 ppb and greater resulted in significant immobility.

Approvals:

J.F. Barbieri 12/1/95
Study Director/Date
J.F. Barbieri

M.T. BenKinney 12/1/95
Supervisor/Date
M.T. BenKinney

C.R. Mackerer 12/1/95
President/Date
C.R. Mackerer

Distribution: Study Director, Liaison, Archives (Original)

STATIC-RENEWAL 48-HOUR ACUTE TOXICITY STUDY OF THE WATER ACCOMMODATED FRACTION (WAF) OF WHOLE LIGHT ALKYLATE PRODUCT TO *Daphnia magna*

STUDY No.: 65907

MATERIAL TESTED:	**Whole Light Alkylate Product**
CRU SAMPLE No.:	**94194**
REQUESTER:	**Petroleum Product Stewardship Council c/o Synthetic Organic Chemical Manufacturing Association 1100 NY Ave., NW, Suite 1090 Washington, D.C. 20005**
STUDY PERFORMED BY:	**Stonybrook Laboratories Inc. 311 Pennington-Rocky Hill Road Pennington, N.J. 08534**
STUDY INITIATION DATE:	**July 22, 1994**
EXPERIMENTAL START DATE:	**November 9, 1994**
EXPERIMENTAL TERMINATION DATE:	**December 1, 1994**

Compliance Statement

Study No. 65907

This study was conducted according to the USEPA Toxic Substances Control; Good Laboratory Practice Standards. 40 CFR Part 792, except as noted below; the final report fully and accurately reflects the raw data generated in the study.

Exceptions to GLPs:

1. The test material, Whole Light Alkylate Product, was not characterized and stability analysis was not performed at this facility.

2. Some data entries were made late. These late entries were indicated as such.

3. Some equipment logs were not up to date at the time of the study.

L.F. Bentivia/M.B.K. 12/1/95

Study Director Date

STONYBROOK LABORATORIES INC.

QUALITY ASSURANCE STATEMENT

Study Number: 65907

Title of Study: Static-Renewal 48-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to *Daphnia magna*

Listed below are the dates that this study was reviewed by the Quality Assurance Unit and the dates that the findings were reviewed by the Study Director and Management.

DATE(S) OF QA REVIEW	PHASE OF STUDY	DATE(S) REVIEWED BY STUDY DIRECTOR	DATE(S) REVIEWED BY MANAGEMENT
11/17/94	PROTOCOL REVIEW	2/3/95	2/25/95
11/23/94	IN-PROCESS INSPECTION	2/19/95	3/1/95
3/31/95	FINAL REPORT AUDIT	4/3/95	5/20/95

Lisa Mick
Manager, Quality Assurance

8/25/95
Date

Amy Wagstaff
PRINCIPAL INVESTIGATOR

J.F. Barbieri/MTB
STUDY DIRECTOR 12/1/85

* no longer with company
MTB 12/1/85

DISTRIBUTION:

Liaison:	C.A. Schreiner, Ph.D.
Principal Investigator:	A.L. Wagstaff, B.A.
Study Director:	J.F. Barbieri, B.S.
Supervisor:	M.T. BenKinney, M.S.
President, Stonybrook Laboratories Inc.:	C.R. Mackerer, Ph.D.
Archives	

Additional Personnel Involved with the Study

N.L. Afonina : Laboratory Technician

A.L. Crawford : Culturist

J.S. Gross : Laboratory Technician

A.L. McClurg : Laboratory Technician

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SUMMARY:

A static-renewal 48-hour toxicity study was conducted November 21-23, 1994 to determine the acute toxicity of Whole Light Alkylate Product to *Daphnia magna*, a representative freshwater invertebrate species. Test daphnids were exposed to individual water accommodated fractions (WAF) of the poorly water-soluble test material at nominal concentrations of 9 ppm, 18 ppm, 35 ppm, 70 ppm, and 140 ppm (w/v, based on density). Nominal test concentrations are based on the loading rate, or amount of test material added to make each WAF. Test solutions were renewed daily during the study. Water quality parameters of pH, temperature, dissolved oxygen (D.O.), conductivity, alkalinity, and hardness were measured for the test chambers throughout the study.

Samples of the control and exposure concentrations were collected at 0, 24, and 48 hours and quantitatively analyzed using purge-and-trap/gas chromatography (GC). The concentrations were quantitated using standard Whole Light Alkylate Product component standards. Measured test concentrations are based on the total concentration of all analytes. Test material retention from the static-renewal procedure ranged from 48.5-66.7%, producing consistent exposure of the test daphnia to Whole Light Alkylate Product throughout the study.

The toxicity of the test material was evaluated on the basis of EC_{50} determinations at 24 and 48 hours. The term EC_{50} used in this report refers to the concentration immobilizing 50% of the test population after a specified exposure period. The computer-estimated 48-hour EC_{50} for Whole Light Alkylate Product was 32 ppm, based on nominal concentrations, and 556 ppb, based on measured concentrations. The 48-hour no observed effect concentration (NOEC), based on nominal concentrations, was 18 ppm, since exposure to concentrations of 35 ppm and greater resulted in significant immobility. The 48-hour no observed effect concentration (NOEC), based on measured concentrations, was 339 ppb, since exposure to concentrations of 596 ppb and greater resulted in significant immobility.

INTRODUCTION:

The objective of this study was to determine the acute toxicity of Whole Light Alkylate Product to aquatic organisms by evaluating its effect on *Daphnia magna*, a representative freshwater invertebrate species. Daphnids were selected since they are a freshwater test species recommended in U.S. EPA (1) regulations. Static-renewal testing of the water accommodated fraction (WAF) was chosen as the most appropriate study design, due to the volatile nature of the test material. Under WAF exposure conditions, toxic effects from the soluble components of the test material are evaluated.

The analytical standards chosen to evaluate the WAF of Whole Light Alkylate Product were selected as representative of the alkane and cycloalkane constituents which account for 68% of the test material. These constituents were expected to be found in the highest concentrations in the WAF and account for most, if not all, of the toxicity measured during the study.

In acute toxicity tests, the most commonly used adverse effect criterion is death of the organism. Due to the difficulty in determining death of Daphnia, the criterion for identification of adverse effects will be immobilization. Immobilization data collected during the study are used to calculate an EC_{50} (concentration affecting 50% of the test population after a specific time period which typically is 48 hours).

METHODS AND MATERIALS:

Test Organisms:

The *Daphnia magna* used in the study were obtained from an in-house culture which has been maintained in the laboratory since January, 1994. The primary culture originated from Aquatic Research Organisms, Hampton, NH, who obtained their culture from the Environmental Protection Agency Laboratory in Cincinnati, OH. Individual daphnids were cultured in 50 mL plastic cups held in a temperature controlled incubator (20 ± 2 °C) on a 16-hour light/8-hour dark cycle following acceptable culturing techniques (2,3,4). The water source was aged Mobil Technical Center (MTC) well water (Table 1). During culturing, the daphnids were fed a vitamin-enriched solution of Yeast/Trout Chow/Cerophyl (YTC) and green algae (*Selenastrum capricornutum*). Only daphnids less than 24 hours old were used in the study. The daphnids were not fed during conduct of the study. Since individual identification of the test daphnids is not possible, daphnids were collected and arbitrarily added to each test chamber.

Test System:

The daphnids were exposed to individual WAFs of Whole Light Alkylate Product. Generation of the WAFs was provided via modification of the procedure used by Anderson, et al (5). Approximately twenty-five hours prior to test initiation, six individual 1 liter WAF aspirator bottles were set up. A stir bar and 1.2 liters of test water were placed into each bottle. A 1 liter bottle filled to the neck (instead of the normal shoulder height) can hold 1.2 liters. The bottles were filled to neck height to minimize volatility. A measured amount of Whole Light Alkylate Product (nominal concentration), calculated for each exposure concentration, was pipetted into each bottle below the solution surface. All aspirator bottles were capped tightly with teflon lined stoppers and the bottle top and stopper were covered with parafilm. The bottles were also covered completely with aluminum foil to retard any possible photodecomposition. The stirring speed of the bottles was adjusted to produce a less than 25% vortex. The solutions stirred for approximately 24 hours, and then were allowed to settle for approximately 45 minutes. After the stirring/settling period, the aqueous phase (WAF) was drained from the bottom spout of each aspirator bottle. Five samples were collected from each individual WAF. Two test replicates contained test organisms, which were added within one hour of WAF addition. The third sample contained no organisms, and was used for initial water quality measurements. Two samples were collected for initial chemical analysis. The solution in each test container was renewed daily during the study. The renewal concentrations were produced in the same manner as the initial concentrations. The test daphnia were transferred from the final concentrations into the corresponding newly made concentrations. Final water quality measurements were made in the final replicates after test organism transfer.

The Whole Light Alkylate Product static-renewal toxicity study was conducted in labeled 237 ml glass jars containing 237 ml of WAF solution. The test jar labeling included the study number, CRU number, test date, concentration, group number, replicate letter, and species designation. The water source for the study was aged MTC well water. The test exposure chambers were held in an incubator at 20 ± 1 °C. Daily temperature readings were taken within the incubator and documented. These internal readings indicated that the incubator was maintained at the desired test temperature. The photoperiod during testing was the same as that provided during acclimation (16-hr light/8-hr dark, fluorescent lighting). All test chambers were sealed tightly with a teflon lined jar lid to minimize evaporation and volatilization of the test material.

Test Material:

The test material, Whole Light Alkylate Product, was dispensed by Stonybrook Laboratory's Chemical Repository Unit (CRU) from a homogeneous sample obtained from the sponsor. As reported in the Product Physical and Chemical Data (PPCD) sheet, Whole Light Alkylate Product (CRU No. 94194) consists entirely of Light Alkylate Naphtha. It was received as a liquid. The stability, identity, strength, purity, and composition or other characteristics which identified the test material was the responsibility of the sponsor. The concentrations used in this study were prepared by pipetting known quantities into each aspirator bottle beneath the surface on a weight to volume basis, based on the density (0.7 g/ml) of the test material. Following a stirring and settling period, the aqueous phase of each solution was used for its corresponding exposure concentration.

Test Procedure-Biological:

A preliminary test which was not protocol driven was run October 18-20, 1994. The data from this study was not used in the determination of the toxicity of the test material.

A range finding test was run November 9-11, 1994, following the procedure outlined in the protocol. This study was performed using a static renewal procedure, sealed test chambers, and a 24 hour/45 minute stirring/settling period. Test daphnids were exposed to a control and concentration doses of 1.2 ppm, 9.9 ppm, and 99 ppm, evaluated in duplicate. At test termination, no mortality was observed in the control, or in the 1.2 ppm and 9.9 ppm concentrations. Also at 48 hours, nearly total mortality (19 daphnids, 95%) was found in the highest concentration, 99 ppm. Based on these results, a dose range of 9-140 ppm was established for the definitive study.

The 48 hour definitive toxicity study documented in this report was conducted November 21-23, 1994. Exposure was initiated by arbitrarily adding daphnids to test chambers after WAF addition, within an hour of WAF collection. Duplicate groups of 10 daphnids per dose level were tested during the study. *Daphnia magna* were exposed to test exposure chambers consisting of a control and five nominal concentrations of Whole Light Alkylate Product (9 ppm, 18 ppm, 35 ppm, 70 ppm, 140 ppm). The control chambers consisted of the same dilution water, test conditions and test organisms with no added test material. Test organisms were observed daily for immobilization at 1, 3, 6, and 24 hours following study initiation. Due to the difficulty in determining death of daphnids, the criterion for identification of adverse effects was immobilization. Daily observations at 1, 3, and 6 hours were made with the jar lids tightly covered, to prevent volatilization. This procedure was necessary, but decreased the accuracy of the 1, 3, and 6 hour observations. The 24 and 48 hour (day 1, 24 hour) observations were made with the lids removed. Daphnids were considered immobilized if they showed no evidence of swimming or forward motion, even after prodding. Due to the short duration of the study, immobilized daphnids were not removed from the test containers during the study.

Test Procedure-Water Quality:

Water quality parameters of dissolved oxygen (D.O.), pH, and temperature were measured at study initiation and daily in a portion of the freshly-prepared initial sample. These water quality parameters were also taken daily in all final replicate test chambers after Daphnia transfer. Conductivity, alkalinity, and hardness were measured for the control, lowest, middle, and highest concentrations at test initiation and termination. Dissolved oxygen was measured with a YSI Model 57 D.O. Meter with a Model 5739 D.O. probe. The pH was measured with an Orion Model 520A Digital pH/mV Meter with an Orion Model 81-02 Combination pH Electrode. Temperature was measured with a hand-held thermometer, with a stainless steel thermocouple. Conductivity was measured

with a YSI Model 33 Salinity-Conductivity-Temperature Meter. Alkalinity and hardness were measured using titration methods (4).

Test Procedure-Chemical:

Chemical analysis was performed on single 40 ml initial samples of the control and all exposure concentrations at 0 and 24 hours after test initiation, and on single 40 ml final samples of the control and all exposure concentrations at 24 and 48 hours after test initiation. Initial samples were a grab sample from the newly prepared WAF, while the final samples were a composite of two concentration replicates (20 ml each). The samples were collected in 40 ml jars with no head space, and transferred to the Analytical Chemistry group for analysis. The chemical analysis was performed within 14 days of sample collection. The concentration of Whole Light Alkylate Product in each sample (measured concentration) was determined by using purge-and-trap and a gas chromatograph equipped with a flame ionization detector (GC-FID) following the methods validation study (Appendix 2, Study 65969). Details of the method are included in the Appendix. The following components of Whole Light Alkylate Product were quantified: 2,3-dimethyl butane, 2,4-dimethyl pentane, 2,2,4-trimethyl pentane, 2,5-dimethyl hexane, 2,3,4-trimethyl pentane, 2,3,3-trimethyl pentane, and 1-methyl-1-ethyl-cyclopentane. Based on the method validation study, these components represent 68% of the composition of Whole Light Alkylate Product. All chemical analysis was performed by C.W. Chuang of the Analytical Chemistry Group.

Statistical Analysis:

Daily EC_{50} values were calculated on the basis of immobilization data and nominal/measured dose levels. Statistical analysis of the data was calculated by a computer software LC_{50} program developed by Stephan et al. (6). This program statistically calculates the EC_{50} using binomial probability analysis, moving average angle analysis, and probit analysis. The EC_{50} was also calculated using the Spearman-Karber method (7,8). The no observed effect concentration values were calculated using Fisher's exact test (8). These different methods of analyzing the data are used since no one method of analysis is appropriate for all possible sets of data that may be obtained (9). The method selected for analysis of the data present in this report was determined by the characteristics of the data base.

Daily measured dose levels, for each concentration, were a cumulative total of all sample values evaluated between the 0 hour initial sample and the final sample, inclusive, for that time period. Measured dose levels were the cumulative total of all measured test material components, for each concentration. In cases where the measured component levels were below that component's detection limit, a zero value was included in the addition of components. The detection limits were based on the methods validation study (Appendix 2). For the 48 hour time period (all samples), a standard deviation was also calculated. The average measured levels for each time period were used along with corresponding survival data to produce measured EC_{50} and NOEC values. Also for each concentration, all initial sample values were averaged, and all final sample values were averaged. The percent difference between initial and final averages was used to calculate the average percent retention at each exposure period.

Data Storage:

The study was conducted according to the EPA Good Laboratory Practice Standards (40 CFR Part 792) (10). Raw data (Appendix 3) and the original final report are maintained in the Archives of Stonybrook Laboratories in Pennington, New Jersey.

RESULTS:

The EC_{50} values for the 48-hour static-renewal toxicity study of Whole Light Alkylate Product to *Daphnia magna* are summarized in Table 2. The 24 and 48-hour computer-estimated EC_{50} values for Whole Light Alkylate Product, based on nominal concentrations, were 79 ppm and 32 ppm, respectively. The 24 and 48 hour EC_{50} values were 778 ppb and 556 ppb, respectively, based on average measured concentrations (Tables 7 and 8). The 48-hour no observed effect concentration (NOEC), based on nominal concentrations, was 18 ppm, since exposure to concentrations of 35 ppm and greater resulted in significant immobility. The 48-hour no observed effect concentration (NOEC), based on measured concentrations, was 339 ppb, since exposure to concentrations of 596 ppb and greater resulted in significant immobility. All values were determined by binomial probability analysis. Immobilization data for all test chambers are presented in Table 3. Behavioral observations are presented in Table 4.

Water quality parameters of pH, temperature, and dissolved oxygen (D.O.) were measured initially and at each 24 hour observation period. Conductivity, alkalinity, and hardness were measured in the control, lowest, middle, and highest concentrations at test initiation and termination. The water quality values collected during the study are summarized in Tables 5 and 6.

The measured concentrations of Whole Light Alkylate Product in the test chambers were determined by purge-and-trap/gas chromatography (Appendix 1). The concentrations listed in this appendix are based on the coding system identified in the raw data where the first character represents the test concentration group as listed in the protocol; the second character represents either an initial (I) or a final (F) sample; and the third and fourth characters represent the hour of the sampling period. The measured exposure concentrations and calculated averages of the samples collected during the study and the percent retention for average initial and final samples collected during the study are summarized in Tables 7 and 8. The chemical analysis techniques used in this study were developed during the Methods Validation Study (Study 65969). A copy of this study is provided in Appendix 2.

DISCUSSION:

Dissolved oxygen levels remained above 60% saturation. The pH values remained consistent among concentrations. All temperature readings documented in the raw data were within the desired range (20 ± 2 °C). Conductivity, alkalinity, and hardness readings were all within expected levels.

No unusual behavior or immobilization was observed in the controls during the study. By 6 hours after study initiation, all Daphnia in the 140 ppm concentration were immobile. At the 24 hour observation period, partial immobility was observed in the 35 ppm (6 daphnids, 30%) and 70 ppm (7 daphnids, 35%) concentrations. All mobile Daphnia in the 35 and 70 ppm concentrations moved only on prodding or exhibited lethargy. At test termination, no mortality was observed in the 9 or 18 ppm concentrations, with partial mortality observed in the 35 ppm (12 daphnids, 60%) and 70 ppm (13 daphnids, 65%) concentrations. At test termination, all mobile Daphnia in the 35 and 70 ppm concentrations were lethargic. The 48-hour EC_{50} value for the test material was, therefore, 32 ppm, based on nominal exposure concentrations. Based on the average measured concentrations presented in Tables 7 and 8, the 48-hour EC_{50} was 557 ppb.

Samples of the control and exposure concentrations were collected at 0, 24, and 48 hours and quantitatively analyzed using purge-and-trap/gas chromatography (GC). The concentrations were quantitated using standard Whole Light Alkylate Product component standards. Test material retention from the static-renewal procedure ranged from 48.3-66.0%. Daily initial measured concentrations indicated consistent exposure of the test Daphnia to Whole Light Alkylate Product throughout the study.

REFERENCES:

1. Environmental Protection Agency (EPA). 1982. Guidelines and Support Documents for Environmental Effects Testing. EPA 560/6-82-002. Sections EG-1, ES-1.

2. Committee on Methods for Toxicity Tests with Aquatic Organisms. 1975. Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. Environmental Protection Agency (EPA). Ecological Research Series. EPA 660/3-75-009. April, 1975. 61 p.

3. American Society for Testing and Materials. 1991. Standard Guide for Conducting Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians. E729-88a.

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5. Anderson, J.W., Neff, J.M., Cox, B.A., Tatem, H.E. and G.M. Hightower. 1974. Characteristics of Dispersions and Water-Soluble Extracts of Crude and Refined Oils and Their Toxicity To Estuarine Crustaceans and Fish. Marine Biology. 27:75-88.

6. Stephan, C.E., Busch, K.A., Smith, R., Burke, J. and R.W. Andrew. 1978. A Computer Program for Calculating an LC_{50}. U.S. Environmental Protection Agency. Duluth, MN. pre-publication manuscript.

7. Hamilton, M.A., Russo, R.C., and R.V. Thurston. 1977. Trimmed Spearman-Karber Method for Estimating Median Lethal Concentrations in Toxicity Bioassays. Env. Sci. and Tech. 11(7):714-719.

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10 Environmental Protection Agency (EPA). 1989. Toxic Substances Control; Good Laboratory Practice Standards. 40 CFR Part 792. Federal Register. Vol. 54, No. 158.

TABLE 1: Characteristics of MTC Well Water (2 Year Average)

Parameter Measured	Concentration
Dissolved Oxygen	5.2 ppm
pH	7.53
Conductivity	443 µmhos
Total Hardness ($CaCO_3$)	196 mg/L
Alkalinity ($CaCO_3$)	143 mg/L
TSS	<5 mg/L
Ammonia (Distillation as N)	<1 mg/L
Phosphorus (Total as P)	<0.06 mg/L
Sulfate	63 mg/L
COD	<7 mg/L
Cyanide	<0.005 mg/L
Antimony	<0.05 mg/L
Arsenic	<0.01 mg/L
Barium	0.14 mg/L
Beryllium	<0.003 mg/L
Cadmium	<0.001 mg/L
Chromium	<0.002 mg/L
Copper	0.09 mg/L
Iron	<0.1 mg/L
Lead	<0.002 mg/L
Magnesium	18.2 mg/L
Manganese	<0.01 mg/L
Mercury	<0.0002 mg/L
Nickel	<0.05 mg/L
Fluoride	0.1 mg/L
Selenium	<0.004 mg/L
Silver	<0.002 mg/L
Zinc	<0.05 mg/L
TOC	<1 mg/L
NO_3-N	<2 mg/L
Thallium	<0.1 mg/L
Phenols	<0.005 mg/L
Lindane	<0.01 µg/L
Methoxychlor	<0.05 µg/L
Endrin	<0.01 µg/L
Toxaphene	<4 µg/L

TABLE 2: Acute Toxicity of Whole Light Alkylate Product to *Daphnia magna*

	EC_{50}* (95% Confidence Limits)**	
	24 Hours	48 Hours
Nominal	79 ppm (18-140 ppm)	32 ppm (18-140 ppm)
Measured	778 ppb (340-1,264 ppb)	556 ppb (339-1,140 ppb)

	NOEC***	
	24 Hours	48 Hours
Nominal	18 ppm	18 ppm
Measured	340 ppb	339 ppb

* All EC_{50} values were calculated using Binomial Probability Analysis.
** The 95% confidence limits presented above are not actually confidence limits because the LC_{50}s were determined by binomial probability. The limits are statistically sound conservative bounds that are above 95% for the sample size used in this study.
*** All NOEC values calculated using Fisher's Exact Test.

TABLE 3: Number Immobilized During the Acute Toxicity Study of Whole Light Alkylate Product to *Daphnia magna*

Exposure Time	Nominal Concentration (ppm)					
	Control	9	18	35	70	140
Day 0:						
1 hrs.	0/20	0/20	0/20	0/20	0/20	0/20
3 hrs.	0/20	0/20	0/20	0/20	0/20	0/20
6 hrs.	0/20	0/20	0/20	0/20	0/20	20/20
24 hrs.	0/20	0/20	0/20	6/20	7/20	20/20
Day 1:						
1 hrs.	0/20	0/20	0/20	6/20	7/20	20/20
3 hrs.	0/20	0/20	0/20	6/20	7/20	20/20
6 hrs.	0/20	0/20	0/20	6/20	7/20	20/20
24 hrs.	0/20	0/20	0/20	12/20	13/20	20/20

TABLE 4: Behavior Observations During the Acute Toxicity Study of Whole Light Alkylate Product To *Daphnia magna*

Behavior of Survivors	Nominal Concentration (ppm)					
Exposure Time	Control	9	18	35	70	140
Day 0:						
1 hrs.	20N	20N	20N	20L	20L	20L
3 hrs.	20N	20N	20N	20L	20L	20L
6 hrs.	20N	20N	4N,16L	20A	20A	20B
24 hrs.	20N	20N	20N	12P,2L,6B	9P,4L,7B	20B
Day 1:						
1 hrs.	20N	20N	20L	14L,6B	13L,7B	20B
3 hrs.	20N	20N	20N	14L,6B	13L,7B	20B
6 hrs.	20N	20N	20N	14L,6B	13L,7B	20B
24 hrs.	20N	20N	20N	8L,12B	7L,13B	20B

N - Normal
A - Appendage Movement
B - Immobile on Bottom
L - Lethargic
P - Movement on Prodding

TABLE 5: Summary of Initial Water Quality Measurements Taken During the Acute Toxicity Study of Whole Light Alkylate Product to *Daphnia magna*

Test Conc.	Temperature (°C) X*	Range	pH Range	D.O. (mg/l) X	Range
Control	20.3	19.5-21.0	8.04-8.13	8.2	8.0-8.4
9 ppm	20.2	19.4-21.0	8.06-8.14	8.3	8.1-8.4
18 ppm	20.2	19.6-20.8	8.07-8.14	8.4	8.3-8.5
35 ppm	20.2	19.7-20.7	8.08-8.14	8.3	8.2-8.3
70 ppm	20.4	19.9-20.8	8.09-8.14	8.3	**
140 ppm	20.4	19.9-20.9	8.09-8.15	8.3	**

Test Conc.	Conductivity (µmhos)***	Alkalinity (ppm)***	Hardness (ppm)***
Control	390	148	188
9 ppm	385	152	200
35 ppm	385	156	200
140 ppm	390	156	204

* X = Mean
** Reading remained the same throughout the study.
*** Single reading taken at study initiation.

TABLE 6: Summary of Final Water Quality Measurements Taken During the Acute Toxicity Study of Whole Light Alkylate Product to *Daphnia magna*

Test Conc.	Rep.	Temperature (°C) X*	Temperature (°C) Range	pH Range	D.O. (mg/l) X	D.O. (mg/l) Range
Control	A	19.4	19.2-19.5	8.00-8.14	8.2	8.1-8.2
Control	B	19.3	19.2-19.4	8.00-8.15	8.2	8.1-8.3
9 ppm	A	19.3	19.2-19.4	8.03-8.17	8.3	8.2-8.4
9 ppm	B	19.4	19.3-19.4	8.06-8.17	8.3	8.2-8.4
18 ppm	A	19.3	19.2-19.3	8.06-8.17	8.4	8.3-8.4
18 ppm	B	19.2	19.1-19.3	8.08-8.18	8.3	8.1-8.4
35 ppm	A	19.2	**	8.07-8.17	8.4	8.2-8.5
35 ppm	B	19.2	19.1-19.3	8.07-8.18	8.3	8.1-8.5
70 ppm	A	19.3	19.2-19.3	8.08-8.17	8.3	8.2-8.4
70 ppm	B	19.4	19.3-19.4	8.08-8.21	8.2	8.0-8.4
140 ppm	A	19.3	**	8.09-8.19	8.2	8.1-8.3
140 ppm	B	19.3	19.2-19.3	8.09-8.19	8.2	8.1-8.3

Test Conc.	Rep.	Conductivity (umhos)***	Alkalinity (ppm)***	Hardness (ppm)***
Control	A	370	152	200
Control	B	380	144	196
9 ppm	A	375	144	196
9 ppm	B	380	148	188
35 ppm	A	380	144	196
35 ppm	B	385	144	180
140 ppm	A	385	140	200
140 ppm	B	390	144	192

* X = Mean
** Reading remained the same throughout the study.
*** Single reading taken at study termination.

TABLE 7: Measured Exposure Concentrations Determined for the Acute Toxicity Study of Whole Light Alkylate Product to *Daphnia magna*

All values in ppm

Nominal Concentration	0 hr. Initial	24 hr. Final	24 hr. Initial	48 hr. Final
Control	ND	ND	ND	ND
9 ppm	0.274	0.158	0.250	0.188
18 ppm	0.434	0.246	0.383	0.293
35 ppm	0.897	0.454	0.651	0.384
70 ppm	0.856	0.409	1.085	0.528
140 ppm	1.684	0.845	1.266	0.764

ND = Not detected at the method detection limit.

TABLE 8a: Daily Cumulative Averages of the Measured Exposure Concentrations Determined for the Acute Toxicity Study of Whole Light Alkylate Product to *Daphnia magna*

All values in ppm

Nominal Concentration	24 hr. Average	48 hr. (All Samples) Average	Standard Deviation
Control	ND	ND	0.000
9 ppm	0.216	0.219	0.054
18 ppm	0.340	0.340	0.084
35 ppm	0.676	0.597	0.230
70 ppm	0.632	0.720	0.308
140 ppm	1.264	1.140	0.424

TABLE 8b: Initial/Final Averages and Percent Retention of the Measured Exposure Concentrations Determined for the Acute Toxicity Study of Whole Light Alkylate Product to *Daphnia magna*

All values in ppm

Nominal Concentration	Average of Initial Samples	Average of Final Samples	Average % Retention
Control	ND	ND	NC
9 ppm	0.262	0.173	66.0
18 ppm	0.408	0.270	66.0
35 ppm	0.774	0.419	54.1
70 ppm	0.970	0.468	48.3
140 ppm	1.475	0.804	54.5

APPENDIX 1

STONYBROOK LABORATORIES INC.

To: J. F. Barbieri

Date: May 16, 1995

From: C.W. Chuang

CC: M.T. Benkinney

RE: ANALYSIS OF WHOLE LIGHT ALKYLATE PRODUCT IN WATER ACCOMMODATED FRACTION (WAF)

STUDY NO: 65907

The analysis of whole light alkylate product in WAF was performed following a purge-and-trap/gas chromatography procedure recently validated in-house (Study no. 65969). The results are revised as follows:

Table 1. Concentration of analytes in stock solutions prepared at 0 hour

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1I00	0	ND*	ND	ND	ND	ND	ND	ND	----
2I00	9	0.113	0.020	0.063	ND	0.026	0.052	ND	0.274
3I00	18	0.168	0.036	0.091	0.016	0.042	0.073	0.008	0.434
4I00	35	0.402	0.071	0.183	0.018	0.076	0.141	0.006	0.897
5I00	70	0.439	0.063	0.156	0.013	0.063	0.122	ND	0.856
6I00	140	0.822	0.126	0.319	0.038	0.135	0.244	ND	1.684

* ND = not detected at the method detection limit (ref: Study no. 65969).

Table 2. Concentration of analytes of 24-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F24	0	ND	ND	ND	ND	ND	ND	ND	----
2F24	9	0.068	0.009	0.033	ND	0.015	0.033	ND	0.158
3F24	18	0.105	0.017	0.054	ND	0.023	0.047	ND	0.246
4F24	35	0.220	0.030	0.088	ND	0.037	0.079	ND	0.454
5F24	70	0.205	0.028	0.078	ND	0.033	0.063	ND	0.405
6F24	140	0.426	0.059	0.150	0.015	0.066	0.128	0.001	0.845

Table 3. Concentration of analytes in stock solutions prepared at 24 hours

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1I24	0	ND	ND	ND	ND	ND	ND	ND	----
2I24	9	0.098	0.019	0.059	0.004	0.024	0.046	ND	0.250
3I24	18	0.164	0.030	0.084	0.006	0.034	0.065	ND	0.383
4I24	35	0.289	0.050	0.136	0.013	0.055	0.104	0.004	0.651
5I24	70	0.524	0.085	0.211	0.020	0.086	0.157	0.002	1.085
6I24	140	0.633	0.091	0.233	0.026	0.098	0.184	0.001	1.266

Table 4. Concentration of analytes of 48-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F48	0	ND	ND	ND	ND	ND	ND	ND	----
2F48	9	0.078	0.013	0.042	ND	0.018	0.037	ND	0.188
3F48	18	0.131	0.021	0.061	ND	0.026	0.054	ND	0.293
4F48	35	0.176	0.027	0.078	0.005	0.032	0.066	ND	0.384
5F48	70	0.219	0.044	0.115	0.007	0.048	0.095	ND	0.528
6F48	140	0.371	0.056	0.142	0.015	0.061	0.119	ND	0.764

Please call me to discuss the results.

5/16/95

APPENDIX 2

Stonybrook
Laboratories Inc.

Methods Validation for the Analysis of Whole Light Alkylate Product in Water Accommodated Fraction (WAF) Using Purge-and-Trap and GC/FID

Stonybrook Laboratories Inc.
Princeton, NJ

Study Number: 65960

Final Report

STONYBROOK LABORATORIES INC.

REPORT RELEASE

LIAISON: C.A. SCHREINER

STUDY NUMBER: 65969

CRU NUMBER: 94194

TEST ARTICLE: WHOLE LIGHT ALKYLATE PRODUCT

STUDY TITLE: METHODS VALIDATION FOR THE ANALYSIS OF WHOLE LIGHT ALKYLATE PRODUCT IN WATER ACCOMMODATED FRACTION (WAF) USING PURGE-AND-TRAP AND GC/FID

RESULTS:

The development and validation of a purge-and-trap/gas chromatography (PT/GC) method for the analysis of water acclimated fractions (WAF) of whole light alkylate product and the subsequent determination of optimal WAF equilibration times has been completed. The method was developed and validated using seven C6-C8 alkane and cycloalkane standards which represent 68% of the whole light alkylate product. The sensitivity and precision of the assay were validated at the 5 part-per-billion (PPB) level for each of the seven component standards in water. Using this technique, it was determined that the whole light alkylate product freshwater WAF reached equilibrium in approximately 24 hours at a total WAF concentration (sum of n=7 components) of 1.6 parts-per-million (PPM). The saltwater WAF reached equilibrium in approximately 12 hours at a total concentration (sum of n=7 components) of 0.9 PPM.

T.A. Roy 1/30/95
T.A. Roy Date
Study Director

C.A. Schreiner 1/30/95
C.A. Schreiner Date
Vice-President

C.R. Mackerer 2/1/95
C.R. Mackerer
President Date

DISTRIBUTION:
All above, Liaison/C.A. Schreiner, Archives
STUDY NO. 65969

STATEMENT OF COMPLIANCE

The undersigned hereby state that Study No. 65969, Methods Validation for the Analysis of Whole Light Alkylate Product in Water Accommodatated Fraction (WAF) Using Perge-and-Trap and GC/FID, was conducted in compliance with the Good Laboratory Practice Regulations as published in 40 CFR Part 792 Federal Registrar Volume 54-158, 8/17/89 in all aspects with the following exceptions:

The strength, purity and composition or other characteristics to define the test substance was not determined by the testing facility. The methods of synthesis, fabrication, or derivation of the test substance are the responsibility of the sponsor and the data are located at the sponsor's facility.

The purity of purchased reference materials was not determined by the testing facility. It is not known if the purity determination of these chemicals by the supplier were performed under GLPs.

The data acquisition or analysis software on the HP MS DOS operating system used in the study has not been validated in-house.

No bulk inventory usage log was mantained for the test chemicals or analytical standards.

T. A. Roy
Study Director

G. A. Rausina
Study Sponsor

TABLE OF CONTENTS

SUMMARY

The development and validation of a purge-and-trap/gas chromatography (PT/GC) method for the analysis of water acclimated fractions (WAF) of whole light alkylate product and the subsequent determination of optimal WAF equilibration times has been completed. The method was developed and validated using seven C6-C8 alkane and cycloalkane standards which represent 68% of the whole light alkylate product. The sensitivity and precision of the assay were validated at the 5 part-per-billion (PPB) level for each of the seven component standards in water. Using this technique, it was determined that the whole light alkylate product freshwater WAF reached equilibrium in approximately 24 hours at a total WAF concentration (sum of n=7 components) of 1.6 parts-per-million (PPM). The saltwater WAF reached equilibrium in approximately 12 hours at a total concentration (sum of n=7 components) of 0.9 PPM.

EXPERIMENTAL

EXPERIMENTAL DESIGN SUMMARY:

Seven C6-C8 alkanes and cycloalkane, which represent 68% of whole light alkylate product, were selected as the monitored analytes for the in-house method validation. The analyte in methanol solution was spiked into 5 mL deionized water. The aqueous solution were loaded into the purge-and-trap sparger by a Luer Lock syringe. The analytes were then purged out by helium from the aqueous phase to the vapor phase at ambient temperature. The vapor was transferred and consequently trapped in a sorbent tube. After the purge was completed, the sorbent tube was then backflushed and heated. The analytes were swept by helium onto the head of the GC column where the separation and detection took place. The evaluations included measuring each compound's response sensitivity, reproducibility, and purge efficiency. Once the analytical procedure had been verified, a WAF of Whole Light Alkylate Product was generated and evaluated at different time intervals to demonstrate the suitability of the proposed WAF generation procedure.

TEST SUBSTANCES:

ANALYTE NAME	CRU #	LOT #	EXPIRATION	PURITY
2-methylbutane (isopentane)	94570	03859DG	9/99	99%
2,3-dimethylbutane	94565	LA-44304	9/99	99%
2,4-dimethylpentane	94565	LA-44304	9/99	99%
2,5-dimethylhexane	94565	LA-44304	9/99	99%
2,2,4-trimethylpentane	94565	LA-44304	9/99	99%
2,3,4-trimethylpentane	94565	LA-44304	9/99	99%
hexane (surrogate)	110-54-3*	42H06471	1/99	99%
2,3,3-trimethylpentane	94591	244X-5S	10/99	99%
1-methyl-ethylcyclopentane	94590	2360	10/99	99%

*CAS Number

Chemical purity and stability data for reference standards purchased commercially were provided by the suppliers (Supelco, Sigma, Wiley, API Standard Reference Materials). The data provided by the suppliers is archived with the raw data.

APPARATUS AND REAGENTS:

Syringe--5 mL gas-tight glass with Luer Lock.
Micro syringes--10 µL, 25 µL, 50 µL, 100 µL, and 250 µL.
GC vials--Glass with Teflon-lined screw caps.
Volumetric flasks--Variable volume size with ground-glass stoppers.
Analytical balance--0.0001 g.
Methanol--HPLC grade.

Secondary working standard mixes--Two standard mixes of the eight whole light alkylate component alkanes plus hexane were prepared by mixing their individual stock standard in methanol for a concentration of 100 µg/mL: mix I: isopentane, 2,3, 3-trimethypentane, and 1-methyl-1-ethylcyclopentane and mix II contained the remaining 5 analytes plus the surrogate, hexane.

Calibration standards--Five levels of standards (approximately 1, 5, 10, 25 and 50 µg/mL) were prepared from the secondary working standard mixes.

Spiking surrogate standard--An approximately 10 µg/mL of hexane was prepared in methanol from the stock standard. This solution was spiked in all blanks, spikes, and samples prior to analysis.

Storage and handling precautions --All solutions (except stock standards) were stored at 4°C and labeled with study number, names, concentrations, and expiration date. All solutions will be disposed of upon release of the final report

PROCEDURE:

Set up the acquisition sequence on the Waters chromatography data system.

A 5 mL Luer Lock syringe is filled to overflowing with deionized water which has also been heated to boiling to remove residual volatile organics. The plunger is replaced and the water compressed to the 5 mL mark. The plunger is pulled back slightly to allow for the addition of 5 µL of calibration standard or spiking surrogate standard. After the solution is loaded to the P&T, press START on the LSC 2000 front panel to start the purge-and-trap procedure.

Initial calibration - Run five levels of calibration standards following the procedure described above and calculate the response factor (RF) of the individual analytes based on equation (I):

$$RF = A_S/C_S \qquad (I)$$

where:

A_S: peak area count of analyte

C_S: amount in nanograms (e.g., 5 µL of a 1.0 µg/mL solution = 5 ng) of the calibration standard injected into the syringe

Calculate the average response factor (RF_{ave}) and standard deviation (SD) of five-level calibration standards. Calculate the relative standard deviation (%RSD = (SD/RF_{ave}) x 100) of the calibration using Microsoft Excel (version 4.0). If %RSD is < 20%, then the RF_{ave} of the analytes is used for quantitation. If %RSD > 20%, the first degree linear regression (forced through zero) with r> 0.99 is used for quantitation (re: quantitative analysis section).

Sample analysis - The analysis follows the steps described above. Samples were analyzed only once using one of two duplicate sample vials except when a need for further confirmation arose or when dilutions were required to bring the response of the analytes within the range of the calibration standards. The duplicate sampling vials were used in these cases.

RESULTS & DISCUSSION

METHOD EVALUATION/VALIDATION:

The use of the PT/GC technique for the analysis of whole light alkylate product WAF was based on a review of the test article composition and the anticipated composition of the WAF. The use of PT/GC runs throughout the EPA analytical methods series for drinking water (500), municipal/industrial effluent water (600) and wastewater (8000). The method has been tentatively validated for the analysis of gasoline range organics (GRO) in the last year and drafts of the method were made available by the Office of Solid Waste (OSW) prior to the expected promulgation in late 1994.

Six alkanes and one cycloalkane were selected (representing 68% of the components of the test material) for the in-house evaluation/validation. Hexane was chosen as the surrogate. The EPA procedure for the evaluation of method performance is an appropriate standard by which to assess in-house method validation. Determination of the method detection limit (MDL), limit of detection (LOD) and limit of quantitation (LOQ) provide an excellent measure of the sensitivity and precision of the procedure. MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The LOD is the lowest concentration that can be determined statistically differently from the blank. LOD is numerically defined as three times the standard deviation from replicate measurements of standard. LOQ is the level above which quantitative results may be obtained and is numerically defined as ten times the standard deviation from replicate measurements of standards. The LOD, LOQ and MDL were determined from replicate measurements of the analytes and surrogate in water at 5 PPB. In general, the per component MDL was slightly below 5 PPB. The LOD, LOQ and MDL for each of the compounds is reported in table I.

WAF GENERATION AND EVALUATION:

Two types of WAF were generated to evaluate the affect of mixing and headspace on final WAF concentration. The concentration of test article components was significantly higher (factor of 2) in the "minimal headspace" type WAF as compared to the "maximum phase interface" type WAF. Table II reports the time course of WAF concentration for the individual and summed seven analytes monitored for both freshwater (through 72 hours) and saltwater (through 48 hours). The surrogate recoveries, which were essentially quantitatve, are also reported for each WAF sample analyzed.

WAF concentration of test material peaked at approximately 12 hours in saltwater (0.9 PPM) and 24 hours in freshwater (1.6 PPM) using the "minimal headspace"WAF generation procedure. This can be seen more clearly in Figure 1 where the "Total" column data in table II for freshwater and saltwater WAF concentrations are plotted vs time of sampling in a histogram format. Figure 2 plots the individual component concentrations for freshwater and saltwater WAF vs sampling time and shows that the relative concentration of the individual test article WAF components is largely maintained over the mixing period. Figures 3 and 4 compare the 24 hour WAF concentration of the test article components with the actual concentration of the components in the test article. These experimentally observed results can be predicted with a reasonable degree of accuracy if the water solubility or octanol/water partition coefficients of the components are taken into consideration.

Table 1

Summary Sheet for LOD, LOQ and MDL Determinations for Whole Light Alkylate Product WAF Components and Surrogate

Peak#	Compound	Rt. (min.)	Area count						
			Run1	Run2	Run3	Run4	Run5	Run6	Run7
1	2,3-dimethylbutane	8.065	37422	31896	36712	34842	30650	20817	46749
2	benzene (surr)	8.850	35696	30159	34788	34045	28001	19625	46398
3	2,4-dimethylpentane	9.600	41098	34678	40270	37909	33107	22357	52058
4	2,2,4-trimethylpentane	11.420	43558	36274	41736	41538	36182	25682	54862
5	2,5-dimethylhexane	12.750	40754	34303	39308	40101	34222	24153	51361
6	2,3,4-trimethylpentane	13.480	41512	35834	41050	42265	37296	25760	53336
7	2,3,3-dimethylpentane	13.680	41454	36507	41116	42461	38096	35948	41716
8	1-methyl-1-ethylcyclopentane	15.115	46856	42044	46061	49500	44715	45595	47700

Peak#	Compound	Rt. (min.)	PFSCL ODLOQ R1	PFSCL ODLO QR2	PFSCL ODLO QR3	PFSCL ODLOQ R4	PFSCL ODLOQ R5	PFSCL ODLO QR6	PFSCL ODLO QR7	RF(ave)	Std. Dev.	%RSD	Std. Dev. (ppb)	LOD (ppb)	LOQ (ppb)	t value*	MDL (ppb) =Std. Dev. x t
			Response factor=Area count/5 (ng)														
1	2,3-dimethylbutane	8.065	7484.4	6379.2	7342.4	6968.4	6130.0	4163.4	9349.8	6831.1	1573.9	23.0	1.2	3.5	12	3.71	4.3
2	benzene (surr)	8.850	7139.2	6031.8	6957.6	6809.0	5600.2	3925.0	9279.6	6534.6	1637.6	25.1	1.3	3.8	13	3.71	4.6
3	2,4-dimethylpentane	9.600	8219.6	6935.6	8054.0	7581.8	6621.4	4471.4	10411.6	7470.8	1805.8	24.2	1.2	3.6	12	3.71	4.5
4	2,2,4-trimethylpentane	11.420	8711.6	7254.8	8347.2	8307.6	7236.4	5136.4	10972.4	7995.2	1774.6	22.2	1.1	3.3	11	3.71	4.1
5	2,5-dimethylhexane	12.750	8150.8	6860.6	7861.6	8020.2	6844.4	4830.6	10272.2	7548.6	1656.2	21.9	1.1	3.3	11	3.71	4.1
6	2,3,4-trimethylpentane	13.480	8302.4	7166.8	8210.0	8453.0	7459.2	5152.0	10667.2	7915.8	1653.5	21.0	1.0	3.1	10	3.71	3.9
7	2,3,3-dimethylpentane	13.680	8290.8	7301.4	8223.2	8492.2	7619.2	7189.6	8343.2	7922.8	538.9	6.80	0.34	1.0	3.4	3.71	1.3
8	1-methyl-1-ethylcyclopentane	15.115	9371.2	8408.8	9212.2	9900.0	8943.0	9119.0	9540.0	9213.5	471.2	5.11	0.26	0.77	2.6	3.71	0.95

* t value at 99% confidence interval

Table II
Continued

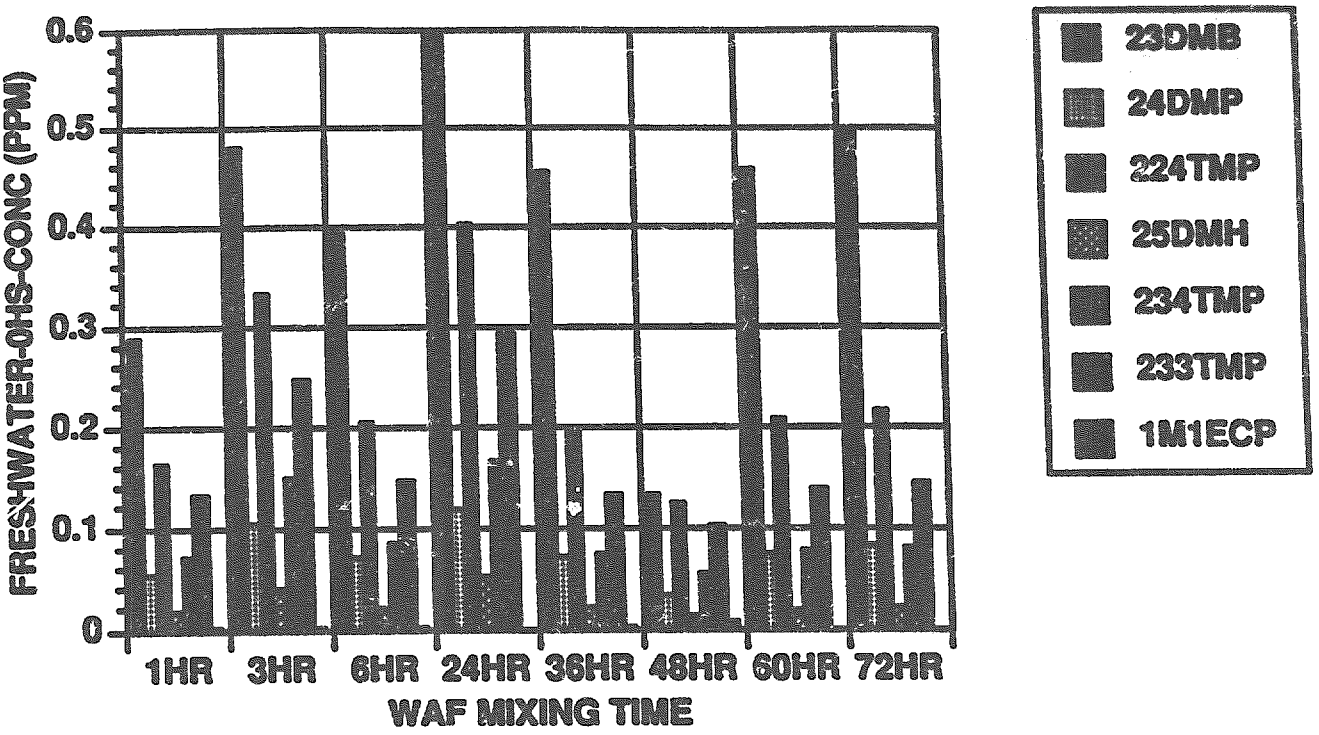
		2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total	hexane (surr) recovery (%)
	RF(ave)	*7524.2*	*8025.4*	*8569.9*	*8495.5*	*8756.4*	*8818.6*	*9204.0*		*7378.6*
Data file	DF*									
36FW1A	20	0.455	0.072	0.197	0.022	0.073	0.133	0.002	0.953	111
36SW1A	10	0.372	0.056	0.145	0.016	0.055	0.101	0.002	0.747	114
36FW2A	20	0.206	0.041	0.128	0.014	0.055	0.103	0.000	0.547	109
36SW2A	10	0.174	0.036	0.117	0.014	0.052	0.100	0.000	0.492	102
48FW1A	20	0.132	0.031	0.125	0.016	0.054	0.102	0.006	0.465	110
48SW1A	10	0.327	0.049	0.134	0.013	0.050	0.099	0.000	0.672	109
48FW2A	20	0.331	0.062	0.186	0.019	0.074	0.140	0.000	0.812	81
48SW2A	10	0.166	0.033	0.100	0.012	0.044	0.084	0.000	0.438	114
60FW1A	20	0.456	0.074	0.206	0.021	0.076	0.138	0.000	0.972	102
60FW2A	20	0.242	0.055	0.184	0.024	0.082	0.146	0.000	0.733	109
72FW1A	20	0.500	0.081	0.216	0.023	0.079	0.143	0.000	1.042	109
72FW2A	20	0.222	0.046	0.142	0.016	0.059	0.110	0.000	0.595	101

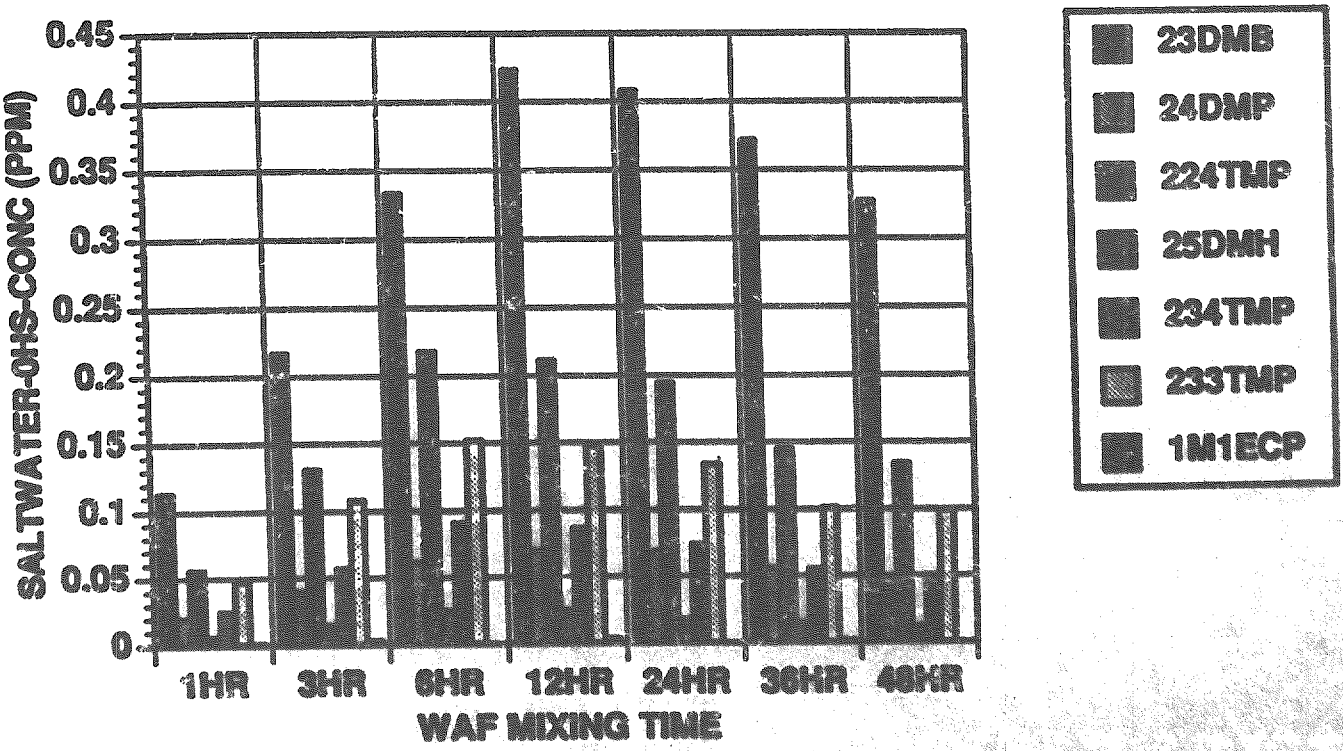
*dilution factor

Data file format - e.g., 36FW1A = 36 hour collection time, freshwater, type "1" WAF (see experimental section), "A", first of two (duplicate) samples collected at the indicated time point.

Figure 2

Individual Monitored Component Concentrations in Whole Light Alkylate Product Freshwater and Saltwater WAFs over 48-72 Hours





WAF GENERATION AND EVALUATION:

Two types of WAFs of Whole Light Alkylate Product were evaluated to demonstrate equilibrium and maintenance of test material. A WAF prepared with freshwater was evaluated at 0,1,3,6,24,36,48,60 and 72 hours after preparation while a WAF prepared with saltwater was evaluated at 0,1,3,6,12,24,36 and 48 hours after preparation. The WAFs were generated following modification of the procedure used by Anderson, et al (1974, Marine Biol., 27: 75-88). Two WAFs were prepared, using each water type, containing 50 ppm of Whole Light Alkylate Product. One WAF of each water type was prepared in a bottle filled to the neck to minimize headspace ("XXX1X" sample designation, e.g. sample "3FW2B" is a 3-hour, freshwater, type 1 WAF, the second of duplicate samples collected), while the second WAF of each water type was prepared in a bottle filled to the shoulder to maximize product-water contact ("XXX2X" sample designation). Duplicate samples were collected from each bottle (except for time zero "XXX2X" series) at the specified time periods, with one sample analyzed using the methodology determined from the in-house validation and the other sample acting as a backup. All samples were collected in 40 ml glass vials with no headspace. The concentration in each flask was quantified to evaluate the consistency of the WAF with time, water type and stirring procedure.

GOOD LABORATORY PRACTICES:

This study was conducted according to the EPA Good Laboratory Practice Standards outlined in 40 CFR Part 160, Federal Register Vol. 54, No.158, 8/17/89.

Test Substance(s) Characterization - The methods of synthesis, fabrication, and/or derivation of the test materials is the responsibility of the sponsor. In addition, the stability, identity, strength, purity and composition of other characteristics which identify the test materials are the responsibility of the sponsor. The test article data are located at the sponsor's facility.

Chemical purity and stability data for reference and control standards purchased commercially, with the exception of 2,3,3-trimethylpentane and 1-methyl-ethylcyclopentane, were provided by the suppliers (Supelco, Sigma). The latter two compounds were assayed for purity at Stonybrook Laboratories Inc. These data and those provided by the suppliers are archived with the raw data.

RECORDS MAINTAINED:

The study file contains but is not limited to the following records or verified copies of:

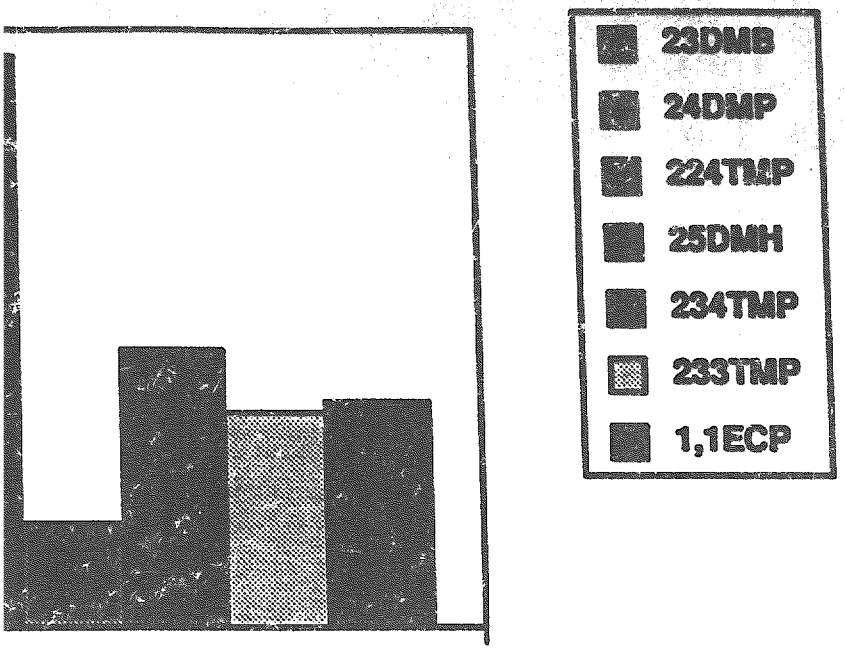
- Notice of Intent to Initiate Study
- Request for Testing
- Sponsor Protocol Amendment Approval Memo
- Study Protocol and Amendments
- Technical Personnel Records
- Reagents and Equipment Inventory
- Chemical Repository Unit (CRU) Dispensing Records
- Study Notebook Records

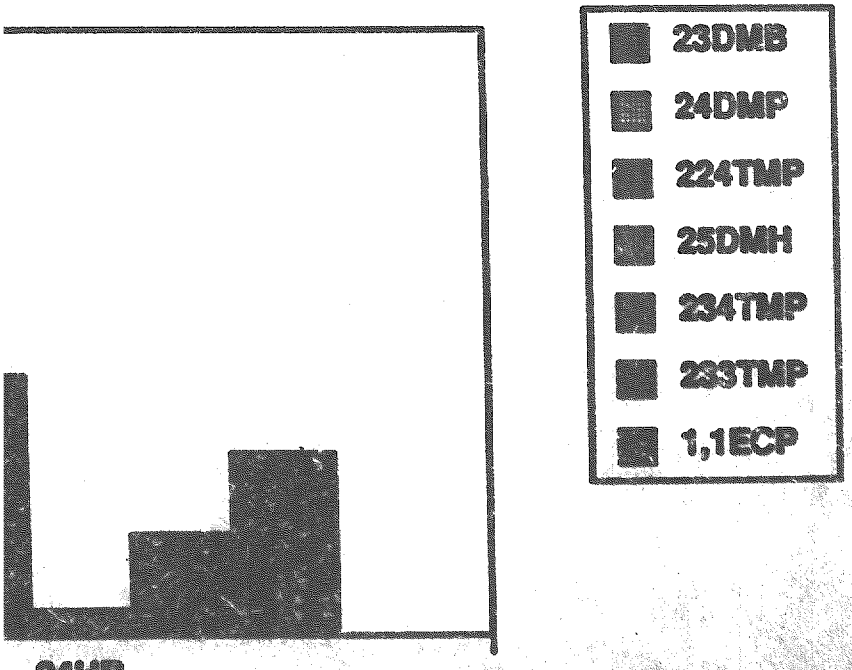
Figure 4

Study No. 65969

ght Alkylate Product Alkane Concentrations In
Their 24 Hour WAF Concentration (Saltwater)

DATA



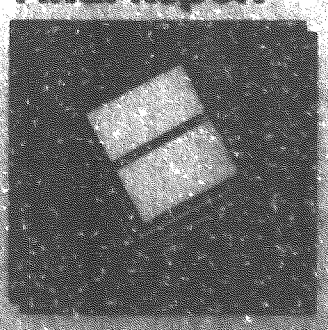


Stonybrook
Laboratories Inc.

Static 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to a Freshwater Alga, *Selenastrum capricornutum*

Stonybrook Laboratories Inc.
Princeton, NJ

Final Report



Study Number 65609

STONYBROOK LABORATORIES INC.
REPORT RELEASE

TO STUDY DIRECTOR/LIAISON: C.A. Schreiner
STUDY NUMBER: 65909
CRU NUMBER: 94194
SAMPLE NAME: Whole Light Alkylate Product
STUDY TITLE: Static 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to a Freshwater Alga, *Selenastrum capricornutum*

REQUESTING DIVISION: Petroleum Product Stewardship Council

RESULTS: EC_{50} 45 ppm for Whole Light Alkylate Product (Nominal)
EC_{50} 741 ppb for Whole Light Alkylate Product (Measured)

A static 96-hour toxicity study was conducted January 12-16, 1995 to determine the acute toxicity of Whole Light Alkylate Product to *Selenastrum capricornutum*, a representative freshwater green algae. Test algae were exposed to individual water accommodated fractions (WAFs) of the poorly water-soluble test material at nominal concentrations of 18 ppm, 70 ppm, 146 ppm, 292 ppm, and 1,157 ppm (w/v, based on density). Nominal test concentrations are based on the loading rate, or amount of test material added to make each WAF. Test solutions were not renewed during conduct of the study, although destructive sampling of all concentrations was done daily. The pH was measured in the test chambers at daily intervals in the replicates being removed from testing (destructive sampling).

Samples of the control and all exposure concentrations were collected at 0, 24, 48, 72, and 96 hours and quantitatively analyzed using purge-and-trap/gas chromatography (GC). The concentrations were quantitated using standard Whole Light Alkylate Product component standards. Measured test concentrations are based on the total concentration of all analytes. Test material remaining in exposure solutions from the static procedure ranged from 52.2 to 81.4% at 24 hours, to 16.8 to 19.8% at 96 hours. Although volatility losses increased throughout the study, daily comparison of the percent of the test material remaining in each concentration stayed comparatively consistent.

The toxicity of the test material was evaluated on the basis of EC_{50} determinations at 24, 48, 72, and 96 hours. The term EC_{50} used in this report refers to the concentration which inhibits 50% of cell growth compared with the control. The computer-estimated 96-hour EC_{50} for Whole Light Alkylate Product was 45 ppm, based on nominal concentrations, and 741 ppb based on average measured concentrations. The 96 hour no observable effect concentration (NOEC), based on nominal concentrations, was 18 ppm, since exposure to concentrations of 70 ppm and higher produced significant cell growth inhibition. The 96 hour no observable effect concentration (NOEC), based on measured concentrations, was 353 ppb, since exposure to concentrations of 1,060 ppb and higher produced significant cell growth inhibition. Subculturing demonstrated that the cell growth inhibition observed during the study was algistatic rather than algicidal, since resumption of cell growth occurred following transfer to fresh media.

Approvals:

J.F. Barbieri/mtb 12/1/95
Study Director/Date
J.F. Barbieri

M.T. BenKinney 12/1/95
Supervisor/Date
M.T. BenKinney

C.R. Mackerer 12/1/95
President/Date
C.R. Mackerer

Distribution: Study Director, Liaison, Archives (Original)

STATIC 96-HOUR ACUTE TOXICITY STUDY OF THE WATER ACCOMMODATED FRACTION (WAF) OF WHOLE LIGHT ALKYLATE PRODUCT TO A FRESHWATER ALGA, *Selenastrum capricornutum*

STUDY No.: 65309

MATERIAL TESTED:	Whole Light Alkylate Product
CRU SAMPLE No.:	94194
REQUESTER:	Petroleum Product Stewardship Council c/o Synthetic Organic Chemical Manufacturing Association 1100 NY Ave., NW, Suite 1090 Washington, D.C. 20005
STUDY PERFORMED BY:	Stonybrook Laboratories Inc. 311 Pennington-Rocky Hill Road Pennington, N.J. 08534
STUDY INITIATION DATE:	July 22, 1994
EXPERIMENTAL START DATE:	November 10, 1994
EXPERIMENTAL TERMINATION DATE:	February 10, 1995

Compliance Statement

Study No. 65909

This study was conducted according to the USEPA Toxic Substances Control; Good Laboratory Practice Standards. 40 CFR Part 792, except as noted below; the final report fully and accurately reflects the raw data generated in the study.

Exceptions to GLPs:

1. The test material, Whole Light Alkylate Product, was not characterized and stability analysis was not performed at this facility.

2. Some data entries were made late. These late entries were indicated as such.

3 Some equipment logs were not up to date at the time of the study.

L. F. Barlow/M-B-K 12/1/95

Study Director Date

STONYBROOK LABORATORIES INC.

QUALITY ASSURANCE STATEMENT

Study Number: 65909

Title of Study: Static 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to Freshwater Alga, *Selenastrum capricornutum*

Listed below are the dates that this study was reviewed by the Quality Assurance Unit and the dates that the findings were reviewed by the Study Director and Management.

DATE(S) OF QA REVIEW	PHASE OF STUDY	DATE(S) REVIEWED BY STUDY DIRECTOR	DATE(S) REVIEWED BY MANAGEMENT
11/18/94	PROTOCOL REVIEW	11/23/94	1/23/95
12/19/94	IN-PROCESS INSPECTION	2/19/95	2/25/95
1/12/95	IN-PROCESS INSPECTION	1/13/95	1/16/95
4/14/95	FINAL REPORT AUDIT	5/16/95	7/18/95

Lisa Muck
Manager, Quality Assurance

7/26/95
Date

J.S. Gross* MTB
PRINCIPAL INVESTIGATOR

J.F. Barbieri* M.B.K. 12/1/85
STUDY DIRECTOR

* no longer with company
MTB 12/1/85

DISTRIBUTION:

Liaison:	C.A. Schreiner, Ph.D.
Principal Investigator:	J.S. Gross, B.S.
Study Director:	J.F. Barbieri, B.S.
Supervisor:	M.T. BenKinney, M.S.
President, Stonybrook Laboratories, Inc.:	C.R. Mackerer, Ph.D.
Archives	

Additional Personnel Involved in the Study

A.L. McClurg : Laboratory Technician

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SUMMARY:

A static 96-hour toxicity study was conducted January 12-16, 1995 to determine the acute toxicity of Whole Light Alkylate Product to *Selenastrum capricornutum*, a representative freshwater green algae. Test algae were exposed to individual water accommodated fractions (WAFs) of the poorly water-soluble test material at nominal concentrations of 18 ppm, 70 ppm, 146 ppm, 292 ppm, and 1157 ppm (w/v, based on density). Nominal test concentrations are based on the loading rate, or amount of test material added to make each WAF. Test solutions were not renewed during conduct of the study, although destructive sampling of all concentrations was done daily. The pH was measured in the test chambers at daily intervals in the replicates being removed from testing (destructive sampling).

Samples of the control, 18 ppm, 70 ppm, 146 ppm, 292 ppm, and 1157 ppm concentrations were collected at 0, 24, 48, 72, and 96 hours and quantitatively analyzed using purge-and-trap/gas chromatography (GC). The concentrations were quantitated using standard Whole Light Alkylate Product component standards. Measured test concentrations are based on the total concentration of all analytes. Test material remaining in exposure solutions from the static procedure ranged from 52.2 to 81.4% at 24 hours, to 16.8 to 19.8% at 96 hours. Although volatility losses increased throughout the study, daily comparison of the percent of the test material remaining in each concentration stayed comparatively consistent.

The toxicity of the test material was evaluated on the basis of EC_{50} determinations at 24, 48, 72, and 96 hours. The term EC_{50} used in this report refers to the concentration which inhibits 50% of cell growth compared with the control. The computer-estimated 96-hour EC_{50} for Whole Light Alkylate Product was 45 ppm, based on nominal concentrations, and 741 ppb based on average measured concentrations. The 96 hour no observable effect concentration (NOEC), based on nominal concentrations, was 18 ppm, since exposure to concentrations of 70 ppm and higher produced significant cell growth inhibition. The 96 hour no observable effect concentration (NOEC), based on measured concentrations, was 353 ppb, since exposure to concentrations of 1,060 ppb and higher produced significant cell growth inhibition. Subculturing demonstrated that the cell growth inhibition observed during the study was algicstatic rather than algicidal since resumption of cell growth occurred following transfer to fresh media.

INTRODUCTION:

The objective of this study was to determine the acute toxicity of Whole Light Alkylate Product to aquatic organisms by evaluating its effect on *Selenastrum capricornutum*, a representative freshwater green alga. Algae were selected since they are a freshwater test species recommended in U.S. EPA (1) regulations. Static testing of the water accommodated fraction (WAF) in closed containers with as little headspace as possible was chosen as the most appropriate study design for the test material, since static-renewal techniques are too disruptive for algae. Under WAF exposure conditions, toxic effects from the soluble components of the test material are evaluated.

The analytical standards chosen to evaluate the WAF of Whole Light Alkylate Product were selected as representative of the alkane and cycloalkane constituents which account for 68% of the test material. These constituents were expected to be found in the highest concentrations in the WAF and account for most, if not all, of the toxicity measured during the study.

In acute toxicity tests of this type, the most commonly used adverse effect criterion is an alteration in productivity (growth) with increased test material concentration. Percentage growth compared with control cultures following daily exposure periods is used to calculate an EC_{50} (concentration which inhibits 50% of cell growth compared with the control).

METHODS AND MATERIALS:

Test Organisms:

The freshwater green algae (*Selenastrum capricornutum*) used in this study was purchased from American Type Culture Collection (ATCC Strain 22662), Rockville, MD, in June 1994. The algal cultures were axenic and were grown in 125 mL cotton-plugged Erlenmeyer flasks containing 50 mL of nutrient-enriched test solution. The culture media was sterile AAP media (2), enriched with 515 mg/l of sodium bicarbonate (Table 1). Cultures were transferred every 5-9 days to fresh media. The algal cultures were incubated at 24 ± 2 °C in a temperature controlled chamber oscillated at 100 rpm to keep algal cells in suspension. Continuous illumination was provided from a rack of cool-white fluorescent tubes arranged at the top of the chamber. The algae used in the test were in log-phase growth. All test flasks were inoculated from a common algal stock solution.

Test System:

The algae were exposed to individual WAF solutions of Whole Light Alkylate Product. Generation of the WAF solutions was provided via modification of the procedure used by Anderson, et al (3). Approximately twenty-five hours prior to test initiation, six individual 2 liter aspirator bottles were set up. A stir bar and 2.3 liters of sterilized test media were placed into each bottle. Test media was sterilized by 0.22 µ filtration. A measured amount of Whole Light Alkylate Product (nominal concentration), calculated for each exposure concentration, was pipetted into each bottle. All aspirator bottles were capped with teflon lined stoppers and parafilm. All bottles were completely covered with aluminum foil. The stirring speed of the bottles was adjusted to produce a vortex of less than 25%. The solutions stirred for approximately 24 hours, and then were allowed to settle for approximately one hour. The stirring of the third definitive run of this study had an 8 minute stoppage during the 24 hour stirring period. After the stirring/settling period, the aqueous phase (WAF) was collected from each aspirator bottle. Twelve replicates were prepared from each individual WAF. Each replicate was then inoculated from a common algal culture to contain approximately 1,000 cells/ml. Flasks were filled to allow as little headspace as possible and tightly closed. Each flask was then randomly positioned in the controlled environmental chamber under continuous fluorescent light and 100 rpm oscillation at 24 ± 2 °C. The solution in each flask was not renewed for the duration of the study.

The Whole Light Alkylate Product static toxicity study was conducted in labeled 125 mL Erlenmeyer flasks containing 135 mL final volume, leaving no headspace. The test flasks were labeled with the study number, test initiation date, test concentration, group number, replicate letter, species designation, and exposure time length in hours. The water source for the study was sterile AAP media (2), enriched with 515 mg/l of sodium bicarbonate. The media was adjusted to 7.5 ± 0.1, by adding 0.1 N HCl. The pH was not readjusted after algal inoculation or test material addition. A daily temperature reading was taken in a random replicate of the destructive samples. Daily temperature readings were taken also within the incubator and documented. Cell density of the algal stock culture inoculate was determined prior to study initiation with a hemacytometer cell and a compound microscope.

Test Material:

The test material, Whole Light Alkylate Product, was dispensed by Stonybrook Laboratories' Chemical Repository Unit (CRU) from a homogeneous sample obtained from the sponsor. As reported on the product physical and chemical data (PPCD) sheet, Whole Light Alkylate Product (CRU No. 94184) consists entirely of Light Alkylate

Naphtha. It was received in liquid form. The stability, identity, strength, purity, and composition or other characteristics which identified the test material was the responsibility of the sponsor. The concentrations used in this study were prepared by pipetting known quantities into each aspirator bottle on a weight to volume basis, based on the density (0.7 g/ml) of the test material. Following the stirring and settling period, the aqueous phase of each solution was used for its corresponding exposure concentration.

Test Procedure-Biological:

A range finding closed container study was performed November 10-14, 1994 to assess the toxicity of Whole Light Alkylate Product. This preliminary study consisted of a control and test concentrations of 1.1 ppm, 11.2 ppm, 112 ppm, and 1120 ppm. After 96 hours, cell densities of the exposure concentrations when compared to the control showed significant inhibition (59%) only at the 1,120 ppm concentration. Only slight inhibition was found at the 1.1 ppm (10%) and 112 ppm (15%) concentrations, with relative growth observed at the 11.2 ppm concentration. Based on these results, the definitive study was conducted with a dose range of 70-1,167 ppm.

An initial run of this study was conducted November 28-December 2, 1994, consisting of a control and exposure concentrations of 70 ppm, 146 ppm, 292 ppm, 583 ppm, and 1167 ppm. The test was conducted under appropriate test conditions, except that AAP media without sodium bicarbonate enhancement was used. After 96 hours, cell density counts revealed that all test concentrations produced greater than 50% inhibition when compared to the control. Due to the greater than expected inhibition, the study was rerun. Both the range finder and initial run was conducted with AAP media without sodium bicarbonate enhancement. This omission was also a reason to rerun the study.

A second definitive run was conducted December 16-20, 1994, consisting of a control and concentrations of 18 ppm, 70 ppm, 146 ppm, 292 ppm, and 1,167 ppm. This second run was conducted under appropriate test conditions, including AAP media with sodium bicarbonate enhancement. After 96 hours, the control produced cell growth lower than expected (an average of 7,655 cells/ml). Cell densities were then only counted for the 18 ppm and 1,167 ppm concentrations. Both of these concentrations also produced low densities, 8,889 cells/ml and 3,580 cells/ml, respectively. Due to the unexplainable low cell density of the control, the study was rerun.

The 96 hour definitive toxicity study documented in this report (run 3) was conducted January 12-16, 1995. *Selenastrum capricornutum* were exposed to test exposure chambers consisting of a media control and five nominal concentrations of Whole Light Alkylate Product (18 ppm, 70 ppm, 146 ppm, 292 ppm, 1167 ppm). Each concentration was initiated with twelve replicates, and was inoculated with an algal stock culture to achieve a density of 1,000 cells/ml. The control chambers consisted of the same dilution water, test conditions, and test algae with no added test material. Destructive sampling of three replicates per concentration was conducted daily throughout the study. At each daily sampling period, 5 ml samples from each replicate were fixed with Lugol's iodine solution. The cell density was then determined microscopically for each replicate. After 96 hours, subcultures were set up. One-half (1/2) milliliter aliquots were collected from each replicate flask for all the test groups. The concentration replicate aliquots were combined in a 125 mL Erlenmeyer flask containing 50 mL of fresh algal nutrient media and subcultured under test exposure conditions. The subcultures were visually observed or the cell density was determined after a period of nine days.

Test Procedure-Cell Density Determination:

Cell densities were determined by direct microscopic examination. Sample aliquots were collected from each replicate flask and fixed with Lugol's iodine solution. The fixed solution was added to a hemacytometer cell. Following a settling period in the hemacytometer, algal cell density in each sample aliquot was determined by microscopic

counts using a compound microscope. The algal cell density was determined using standard hemacytometer formulas based on the dilution factor, a numerical constant (10,000) based on the ratio of volume in 1 "large square" in the hemacytometer to 1 ml, and the area of the hemacytometer counted based on large squares. Three counts were made for each replicate flask used per concentration per day. The mean cell density reported for each concentration, therefore, represents the average of nine cell counts. Statistical analyses were based on the percent cell growth or inhibition for each concentration after daily exposure relative to growth in the controls.

Test Procedure-Subculture:

An algistatic growth response results when cell division ceases, but the cells themselves remain viable. An algistatic response can be determined by transferring a portion of each test solution containing algal growth to fresh media without test material. Inclusion of a subculture cell recovery period makes it possible to evaluate the extent of cell damage and adverse population effect following exposure to the test material. One-half milliliter (1/2 mL) aliquots were collected from replicate flasks of the control and all exposure groups at test termination. The exposure concentration replicate aliquots were resuspended in 50 ml fresh media without test chemical, and incubated for a recovery period of nine days duration. The subculture period was conducted from January 16-25, 1995. Visual observation of the culture for signs of growth (green coloration) during the recovery period, although not quantitative, support the hypothesis of cell recovery and resumption of algal cell growth. Visual growth was only noted in the control subculture. All the exposure concentration subcultures were assessed after nine days using microscope counts.

Test Procedure-Physical/Chemical:

The pH of the algae medium was measured and adjusted to 7.5 ± 0.1, using 0.1 N HCl, prior to concentration preparation. The pH of each destructive replicate test solution was measured daily except for one 18 ppm flask which was broken prior to performance of the pH reading. Shaking speed was read off the chamber rpm meter. Temperature was taken daily in one destructive flask of the control. Light intensity in the environmental chamber was measured at test initiation and termination. The pH of the test solutions was measured with an Orion Model 520A Digital pH/mV Meter with an Orion Model 81-02 Combination pH electrode. The daily replicate temperature was taken with a digital thermometer with stainless steel thermocouple. Temperature was also taken with a mercury filled glass thermometer placed in a water-filled flask in the incubator. The light intensity was measured with a DLM2 factory calibrated light meter.

Chemical analysis was performed on 40 ml samples of the control and all exposure concentrations at the 0, 24, 48, 72, and 96 hours after test initiation. The 0 hour sample was from the initial test concentrations, and the 24, 48, 72, and 96 hour samples were taken from final destructive composites from each exposure concentration. The samples were collected in 40 ml vials with teflon septum open top caps with no head space, and transferred to the Analytical Chemistry group for analysis. The concentration of Whole Light Alkylate Product in each sample (measured concentration) was determined by using purge-and-trap and a gas chromatograph equipped with a flame ionization detector (GC-FID) following the methods developed in the methods validation study (Appendix 2, Study No. 65969). Details of the method are included in the Appendix. The following components of Whole Light Alkylate Product were quantified: 2,3-dimethyl butane, 2,4-dimethyl pentane, 2,2,4-trimethyl pentane, 2,5-dimethyl hexane, 2,3,4-trimethyl pentane, 2,3,3-trimethyl pentane, and 1-methyl-1-ethyl-cyclopentane. Based on the method validation study, these components represent 66% of the composition of Whole Light Alkylate Product. All chemical analysis (Appendix) was performed by C.W. Chuang of the Analytical Chemistry Group. The % retention for each daily test concentration was determined by dividing the ppm of material in each final concentration

for that day by the ppm of material in the initial (0 hour) sample for the corresponding concentration.

Statistical Analysis:

The daily EC_{50} and NOEC values were calculated on the basis of percent cell density relative to cell density in the controls for each set of daily destructive samples. EC_{50} and NOEC values were calculated for both nominal and measured concentrations. The measured concentrations were based on the cumulative total of the concentration of the components. In cases where the measured component levels were below that component's detection limit, a zero value was used in the calculations. The detection limits used were determined in the methods validation study (Appendix 2). Statistical analysis of the data was calculated by a computer software program developed by Stephan et al. (4). This program statistically calculates the EC_{50} using binomial probability analysis, moving average angle analysis, and probit analysis. These different methods of analyzing the data are used since no one method of analysis is appropriate for all possible sets of data that may be obtained (5). Cell growth NOEC values were determined using a computerized program of Fisher's exact test (6). The methods selected for analysis of the data present in this report were determined by the characteristics of the data base.

Data Storage:

The study was conducted according to the EPA Good Laboratory Practice Standards (40 CFR Part 792) (7). Raw data (Appendix 3) and the original final report are maintained in the Archives of Stonybrook Laboratories Inc. located in Pennington, New Jersey.

RESULTS:

The daily EC_{50} and NOEC values for the 96-hour static toxicity study of Whole Light Alkylate Product to *Selenastrum capricornutum* are summarized in Table 2. The 24, 48, 72, and 96 hour computer-estimated EC_{50} values for Whole Light Alkylate Product, based on nominal concentrations, were >1,157 ppm, >1,157 ppm, 47 ppm, and 45 ppm, respectively. The 24, 48, 72, and 96 hour computer-estimated EC_{50} values for Whole Light Alkylate Product, based on measured concentrations, were >2,662 ppb, >2,372 ppb, 802 ppb, and 741 ppb, respectively. The 24, 48, 72, and 96 hour no observable effect concentration (NOEC) values, based on nominal concentrations, were 1,157 ppm, 1,157 ppm, 1,157 ppm, and 18 ppm, respectively. The 24, 48, 72, and 96 hour no observable effect concentration (NOEC) values, based on measured concentrations, were 2,662 ppb, 2,372 ppb, 1,974 ppb, and 353 ppb, respectively. All EC_{50} values were calculated by binomial probability analysis, and all NOEC values were determined by Fisher's exact test. Subcultures indicated that growth inhibition was algistatic in all exposure concentrations. The relative cell growth and percent inhibition for each treatment in relation to the control are presented in Table 3.

The measured concentrations of Whole Light Alkylate Product in the test chambers were determined by purge-and-trap/gas chromatography (Appendix 1). The measured exposure concentrations and calculated averages of the samples collected during the study and the percent retention are summarized in Tables 4 and 5. The chemical analysis techniques used in this study were developed during the Methods Validation Study (Study 65969). A copy of this study is provided in Appendix 2.

DISCUSSION:

Illumination of the algal flasks during the study remained at the level of 400 ± 50 ft-candles. The pH of the destructive exposure flasks was consistent among the replicates. Temperature readings were within the acceptable range. The average algal cell growth, after 96 hours, was 5.7 x 10^4 cells/mL in the media control. This growth density was substantially greater than the inoculation concentration of 1.0 x 10^3 cells/mL and confirmed log phase growth in the test population. Control cell densities at 24, 48, and 72 hours were 4.6 x 10^3, 7.2 x 10^3, and 1.8 x 10^4 cells/ml, respectively. These values indicate a lag in growth for the first 48 hours of the study.

Toxicity of Whole Light Alkylate Product to *Selenastrum capricornutum* was assessed as the percent cell inhibition of daily destructive exposures relative to growth in the control flasks. Nominal test concentrations are based on the loading rate, or amount of test material added to make each WAF. The 96-hour computer-estimated EC_{50} for the toxicity of Whole Light Alkylate Product to *Selenastrum capricornutum* was 45 ppm, based on nominal concentrations, and 741 ppb based on average measured concentrations. Average measured concentrations were calculated based on daily measured values. Each daily destructive final composite value was averaged with the corresponding initial concentration 0 hour value to produce the average measured concentration. The no observable effect concentration (NOEC) was 18 ppm, since exposure to concentrations of 70 ppm and higher produced significant cell growth inhibition. Subculture growth was visually evident after nine days in only the control. No visual growth was noted in any of the exposure concentration subcultures at nine days. An actual cell count of these subcultures, however, showed substantial growth, indicating that any inhibition was algistatic.

A notable drop in EC_{50} values is evident between the 24 and 48 hour values and the 72 and 96 hour values (>1,157 ppm as compared to 47 ppm and 45 ppm). This decrease is most likely a result of the lag in control cell density growth at 24 and 48 hours. Since the cell growth was low at 24 and 48 hours, the relative growth affect of the test material on the exposure concentrations was less evident. As the cell growth increased in the control at the 72 and 96 hour periods, the effect of the test material in the exposure flasks was manifested.

Samples of the control and all exposure concentrations were collected at 0, 24, 48, 72, and 96 hours and quantitatively analyzed using purge-and-trap/gas chromatography (GC). The concentrations were quantitated using standard Whole Light Alkylate Product component standards. Measured test concentrations are based on the total concentration of all analytes. Test material remaining in exposure solutions from the static procedure ranged from 52.2 to 81.4% at 24 hours, to 16.8 to 19.8% at 96 hours. This daily decrease would be expected in a non-renewal study of a volatile test material. Although volatility losses increased throughout the study, daily comparison of the percent of the test material remaining in each concentration stayed comparatively consistent.

REFERENCES:

1. Environmental Protection Agency (EPA). 1982. Guidelines and Support Documents for Environmental Effects Testing. EPA 560/6-82-002. Sections EG-8, ES-5.

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TABLE 1: Nutrient Medium Composition (AAP)

Macro nutrient Components	Final Concentration
$NaNO_3$	25.50 mg/L
$MgCl_2 \cdot 6H_2O$	12.17 mg/L
$CaCl_2 \cdot 2H_2O$	4.41 mg/L
$MgSO_4 \cdot 7H_2O$	14.68 mg/L
$K_2HPO_4 \cdot 3H_2O$	1.366 mg/L
$NaHCO_3$	15.01 mg/L

Enhancement Component	Final Concentration
$NaHCO_3$	515.0 mg/L

Micro nutrient Components	Final Concentration
$FeCl_3 \cdot 6H_2O$	159.4 µg/L
$Na_2EDTA \cdot 2H_2O$	300.4 µg/L
H_3BO_3	185.5 µg/L
Na_2SeO_4	1.90 µg/L
$MnCl_2 \cdot 4H_2O$	415.3 µg/L
$ZnCl_2$	3.28 µg/L
$CoCl_2 \cdot 6H_2O$	1.43 µg/L
$CuCl_2 \cdot 2H_2O$	.012 µg/L
$NaMoO_4 \cdot 2H_2O$	7.27 µg/L

TABLE 2: Acute Toxicity of Whole Light Alkylate Product to *Selenastrum capricornutum*

Nominal — All values in ppm

	24 Hours	48 Hours	72 hours	96 Hours
EC_{50}* (95% Confidence Limits)**	>1,157 (NA)	>1,157 (NA)	47 (18-70)	45 (18-70)
NOEC***	1,157	1,157	1,157	18

Measured — All values in ppb

	24 Hours	48 Hours	72 hours	96 Hours
EC_{50}* (95% Confidence Limits)**	>2,662 (NA)	>2,372 (NA)	802 (384-1,036)	741 (353-1,060)
NOEC***	2,662	2,372	1,974	353

* All the EC_{50} values were calculated using Binomial Probability Analysis.
** The 95% confidence limits presented above are not actually confidence limits because the EC50s were determined by binomial probability. The limits are statistically sound conservative bounds that are above 95% for the sample size used in this study.
*** All the NOEC values were calculated using Fisher's exact test.

TABLE 3: Cell Density and % Inhibition Data Collected During the Acute Toxicity Study of Whole Light Alkylate Product to *Selenastrum capricornutum*

Conc.	24 hr. Cell Density (cells/mL)	24 hr. % Inhibition	48 hr. Cell Density (cells/mL)	48 hr. % Inhibition
Control	4.57×10^3	----	7.16×10^3	----
18 ppm	2.72×10^3	40.5	9.75×10^3	-36.2*
70 ppm	3.83×10^3	16.2	1.00×10^4	-39.6*
146 ppm	3.95×10^3	13.5	7.26×10^3	-1.7*
292 ppm	3.95×10^3	13.5	5.93×10^3	17.2
1157 ppm	3.95×10^3	13.5	6.67×10^3	6.9

Conc.	72 hr. Cell Density (cells/mL)	72 hr. % Inhibition	96 hr. Cell Density (cells/mL)	96 hr. % Inhibition
Control	1.83×10^4	----	5.70×10^4	----
18 ppm	1.59×10^4	12.8	5.53×10^4	3.1
70 ppm	6.05×10^3	66.9	1.27×10^4	77.7
146 ppm	1.98×10^3	89.2	3.46×10^3	93.9
292 ppm	2.22×10^3	87.8	1.36×10^3	97.6
1157 ppm	1.73×10^3	90.5	1.60×10^3	97.2

* Test concentration produced cell density greater than the control, therefore number indicates % growth enhancement.

TABLE 4: Measured Exposure Concentrations During the Acute Toxicity Study of Whole Light Alkylate Product to *Selenastrum capricornutum*

All values in ppm

Sample	0 hr. Initial	24 hr. Final	48 hr. Final	72 hr. Final	96 hr. Final
Control	ND*	ND	ND	ND	ND
18 ppm	0.594	0.365	0.206	0.174	0.112
70 ppm	1.814	1.476	0.417	0.358	0.305
146 ppm	2.511	1.499	0.805	1.125	0.498
292 ppm	3.239	1.692	1.505	0.655	0.610
1,157 ppm	3.295	2.028	0.716	0.654	0.612

* ND=None Detected

TABLE 5a: Daily Averages of the Measured Exposure Concentrations Determined for the Acute Toxicity Study of Whole Light Alkylate Product to *Selenastrum capricornutum*

Sample	Average of initial sample taken at 0 hours and: 24 hr. Final	48 hr. Final	72 hr. Final	96 hr. Final
Control	ND*	ND	ND	ND
18 ppm	0.480	0.400	0.384	0.353
70 ppm	1.645	1.116	1.086	1.060
146 ppm	2.005	1.658	1.818	1.504
292 ppm	2.466	2.372	1.947	1.924
1,157 ppm	2.662	2.006	1.974	1.954

TABLE 5b: Percent Retention of the Measured Exposure Concentrations Determined for the Acute Toxicity Study of Whole Light Alkylate Product to *Selenastrum capricornutum*

Sample	Percent Retention between sample taken at 0 hours and: 24 hr. Final	48 hr. Final	72 hr. Final	96 hr. Final
Control	NC**	NC	NC	NC
18 ppm	61.4	34.7	29.3	18.9
70 ppm	81.4	23.0	19.7	16.8
146 ppm	59.7	32.1	44.8	19.8
292 ppm	52.2	46.5	20.2	18.8
1,157 ppm	61.5	21.7	19.9	18.6

*ND=None Detected
**NC=Not Calculable

APPENDIX 1

STONYBROOK LABORATORIES INC.

To: J. F. Barbieri

Date: May 15, 1995

From: C.W. Chuang

CC: M.T. Benkinney

RE: ANALYSIS OF WHOLE LIGHT ALKYLATE PRODUCT IN WATER ACCOMMODATED FRACTION (WAF)

STUDY NO: 65909

The analysis of whole light alkylate product in WAF was performed following a purge-and-trap/gas chromatography procedure recently validated in-house (Study no. 65969). The results are revised as follows:

Table 1. Concentration of analytes in stock solutions prepared at 0 hour

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethy lpentane	2,5-dimethyl hexane	2,3,4-trimethy lpentane	2,3,3-trimethy lpentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1I00	0	ND*	ND	ND	ND	ND	ND	ND	----
2I00	18	0.242	0.043	0.127	0.014	0.054	0.114	ND	0.594
3I00	70	0.852	0.135	0.365	0.042	0.145	0.273	0.002	1.814
4I00	146	1.322	0.178	0.438	0.047	0.180	0.346	ND	2.511
5I00	292	1.857	0.228	0.511	0.053	0.202	0.388	ND	3.239
6I00	1157	2.063	0.216	0.442	0.047	0.185	0.342	ND	3.295

* ND = not detected at the method detection limit (ref: Study no. 65969).

Table 2. Concentration of analytes of 24-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F24	0	ND	ND	ND	ND	ND	ND	ND	----
2F24	18	0.179	0.024	0.065	0.007	0.028	0.062	ND	0.365
3F24	70	0.702	0.107	0.298	0.034	0.116	0.219	ND	1.476
4F24	146	0.838	0.100	0.245	0.025	0.101	0.190	ND	1.499
5F24	292	1.085	0.101	0.219	0.024	0.084	0.179	ND	1.692
6F24	1157	1.375	0.121	0.235	0.025	0.090	0.182	ND	2.028

Table 3. Concentration of analytes of 48-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F48	0	ND	ND	ND	ND	ND	ND	ND	----
2F48	18	0.122	0.013	0.027	0.004	0.011	0.028	0.001	0.206
3F48	70	0.311	0.020	0.037	ND	0.013	0.036	ND	0.417
4F48	146	0.558	0.045	0.082	0.015	0.034	0.071	ND	0.805
5F48	292	0.869	0.105	0.189	0.052	0.091	0.160	0.039	1.505
6F48	1157	0.587	0.034	0.045	ND	0.015	0.035	ND	0.716

Table 4. Concentration of analytes of 72-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1f72	0	ND	ND	ND	ND	ND	ND	ND	----
2F72	18	0.113	0.010	0.020	ND	0.008	0.023	ND	0.174
3F72	70	0.270	0.017	0.030	ND	0.011	0.030	ND	0.358
4F72	146	0.732	0.066	0.132	0.015	0.057	0.123	ND	1.125
5F72	292	0.539	0.028	0.039	ND	0.015	0.034	ND	0.655
6F72	1157	0.557	0.028	0.035	ND	0.009	0.025	ND	0.654

Table 5. Concentration of analytes of 96-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F96	0	ND	ND	ND	ND	ND	ND	ND	----
2F96	18	0.081	0.006	0.010	ND	0.004	0.011	ND	0.112
3F96	70	0.238	0.014	0.023	ND	0.008	0.022	ND	0.305
4F96	146	0.376	0.027	0.034	0.010	0.015	0.029	0.007	0.498
5F96	292	0.511	0.023	0.033	ND	0.012	0.031	ND	0.610
6F96	1157	0.517	0.028	0.034	ND	0.010	0.023	ND	0.612

Please call me to discuss the results.

APPENDIX 2

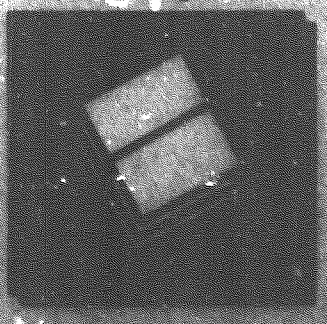
Stonybrook
Laboratories Inc.

Methods Validation for the Analysis of Whole Light Alkylate Product in Water Accommodated Fraction (WAF) Using Purge-and-Trap and GC/FID

Stonybrook Laboratories Inc.
Princeton, NJ

Study Number: 65960

Final Report



STONYBROOK LABORATORIES INC.

REPORT RELEASE

LIAISON: C.A. SCHREINER

STUDY NUMBER: 65969

CRU NUMBER: 94194

TEST ARTICLE: WHOLE LIGHT ALKYLATE PRODUCT

STUDY TITLE: METHODS VALIDATION FOR THE ANALYSIS OF WHOLE LIGHT ALKYLATE PRODUCT IN WATER ACCOMMODATED FRACTION (WAF) USING PURGE-AND-TRAP AND GC/FID

RESULTS:

The development and validation of a purge-and-trap/gas chromatography (PT/GC) method for the analysis of water acclimated fractions (WAF) of whole light alkylate product and the subsequent determination of optimal WAF equilibration times has been completed. The method was developed and validated using seven C6-C8 alkane and cycloalkane standards which represent 68% of the whole light alkylate product. The sensitivity and precision of the assay were validated at the 5 part-per-billion (PPB) level for each of the seven component standards in water. Using this technique, it was determined that the whole light alkylate product freshwater WAF reached equilibrium in approximately 24 hours at a total WAF concentration (sum of n=7 components) of 1.6 parts-per-million (PPM). The saltwater WAF reached equilibrium in approximately 12 hours at a total concentration (sum of n=7 components) of 0.9 PPM.

T.A. Roy 1/30/95
T.A. Roy Date
Study Director

C.A. Schreiner 1/30/95
C.A. Schreiner Date
Vice-President

C.R. Mackerer 2/1/95
C.R. Mackerer
President Date

DISTRIBUTION:
All above, Liaison/C.A. Schreiner, Archives
STUDY NO. 65969

STATEMENT OF COMPLIANCE

The undersigned hereby state that Study No. 65969, Methods Validation for the Analysis of Whole Light Alkylate Product in Water Accommodatated Fraction (WAF) Using Perge-and-Trap and GC/FID, was conducted in compliance with the Good Laboratory Practice Regulations as published in 40 CFR Part 792 Federal Registrar Volume 54-158, 8/17/89 in all aspects with the following exceptions:

The strength, purity and composition or other characteristics to define the test substance was not determined by the testing facility. The methods of synthesis, fabrication, or derivation of the test substance are the responsibility of the sponsor and the data are located at the sponsor's facility.

The purity of purchased reference materials was not determined by the testing facility. It is not known if the purity determination of these chemicals by the supplier were performed under GLPs.

The data acquisition or analysis software on the HP MS DOS operating system used in the study has not been validated in-house.

No bulk inventory usage log was mantained for the test chemicals or analytical standards.

T. A. Roy
Study Director

G. A. Ralusina
Study Sponsor

TABLE OF CONTENTS

SUMMARY

The development and validation of a purge-and-trap/gas chromatography (PT/GC) method for the analysis of water acclimated fractions (WAF) of whole light alkylate product and the subsequent determination of optimal WAF equilibration times has been completed. The method was developed and validated using seven C6-C8 alkane and cycloalkane standards which represent 68% of the whole light alkylate product. The sensitivity and precision of the assay were validated at the 5 part-per-billion (PPB) level for each of the seven component standards in water. Using this technique, it was determined that the whole light alkylate product freshwater WAF reached equilibrium in approximately 24 hours at a total WAF concentration (sum of n=7 components) of 1.6 parts-per-million (PPM). The saltwater WAF reached equilibrium in approximately 12 hours at a total concentration (sum of n=7 components) of 0.9 PPM.

EXPERIMENTAL

EXPERIMENTAL DESIGN SUMMARY:

Seven C6-C8 alkanes and cycloalkane, which represent 68% of whole light alkylate product, were selected as the monitored analytes for the in-house method validation. The analyte in methanol solution was spiked into 5 mL deionized water. The aqueous solution were loaded into the purge-and-trap sparger by a Luer Lock syringe. The analytes were then purged out by helium from the aqueous phase to the vapor phase at ambient temperature. The vapor was transferred and consequently trapped in a sorbent tube. After the purge was completed, the sorbent tube was then backflushed and heated. The analytes were swept by helium onto the head of the GC column where the separation and detection took place. The evaluations included measuring each compound's response sensitivity, reproducibility, and purge efficiency. Once the analytical procedure had been verified, a WAF of Whole Light Alkylate Product was generated and evaluated at different time intervals to demonstrate the suitability of the proposed WAF generation procedure.

TEST SUBSTANCES:

ANALYTE NAME	CRU #	LOT #	EXPIRATION	PURITY
2-methylbutane (isopentane)	94570	03859DG	9/99	99%
2,3-dimethylbutane	94565	LA-44304	9/99	99%
2,4-dimethylpentane	94565	LA-44304	9/99	99%
2,5-dimethylhexane	94565	LA-44304	9/99	99%
2,2,4-trimethylpentane	94565	LA-44304	9/99	99%
2,3,4-trimethylpentane	94565	LA-44304	9/99	99%
hexane (surrogate)	110-54-3*	42H06471	1/99	99%
2,3,3-trimethylpentane	94591	244X-5S	10/99	99%
1-methyl-ethylcyclopentane	94590	2360	10/99	99%

*CAS Number

Chemical purity and stability data for reference standards purchased commercially were provided by the suppliers (Supelco, Sigma, Wiley, API Standard Reference Materials). The data provided by the suppliers is archived with the raw data.

APPARATUS AND REAGENTS:

Syringe--5 mL gas-tight glass with Luer Lock.
Micro syringes--10 µL, 25 µL, 50 µL, 100 µL, and 250 µL.
GC vials--Glass with Teflon-lined screw caps.
Volumetric flasks--Variable volume size with ground-glass stoppers.
Analytical balance--0.0001 g.
Methanol--HPLC grade.

Secondary working standard mixes--Two standard mixes of the eight whole light alkylate component alkanes plus hexane were prepared by mixing their individual stock standard in methanol for a concentration of 100 µg/mL: mix I: isopentane, 2,3, 3-trimethypentane, and 1-methyl-1-ethylcyclopentane and mix II contained the remaining 5 analytes plus the surrogate, hexane.

Calibration standards--Five levels of standards (approximately 1, 5, 10, 25 and 50 µg/mL) were prepared from the secondary working standard mixes.

Spiking surrogate standard--An approximately 10 µg/mL of hexane was prepared in methanol from the stock standard. This solution was spiked in all blanks, spikes, and samples prior to analysis.

Storage and handling precautions --All solutions (except stock standards) were stored at 4°C and labeled with study number, names, concentrations, and expiration date. All solutions will be disposed of upon release of the final report

PROCEDURE:

Set up the acquisition sequence on the Waters chromatography data system.

A 5 mL Luer Lock syringe is filled to overflowing with deionized water which has also been heated to boiling to remove residual volatile organics. The plunger is replaced and the water compressed to the 5 mL mark. The plunger is pulled back slightly to allow for the addition of 5 µL of calibration standard or spiking surrogate standard. After the solution is loaded to the P&T, press START on the LSC 2000 front panel to start the purge-and-trap procedure.

Initial calibration - Run five levels of calibration standards following the procedure described above and calculate the response factor (RF) of the individual analytes based on equation (I):

$$RF = A_S/C_S \qquad (I)$$

where:

A_S: peak area count of analyte

C_S: amount in nanograms (e.g., 5 µL of a 1.0 µg/mL solution = 5 ng) of the calibration standard injected into the syringe

Calculate the average response factor (RF_{ave}) and standard deviation (SD) of five-level calibration standards. Calculate the relative standard deviation (%RSD = (SD/RF_{ave}) x 100) of the calibration using Microsoft Excel (version 4.0). If %RSD is < 20%, then the RF_{ave} of the analytes is used for quantitation. If %RSD > 20%, the first degree linear regression (forced through zero) with r> 0.99 is used for quantitation (re: quantitative analysis section).

Sample analysis - The analysis follows the steps described above. Samples were analyzed only once using one of two duplicate sample vials except when a need for further confirmation arose or when dilutions were required to bring the response of the analytes within the range of the calibration standards. The duplicate sampling vials were used in these cases.

WAF GENERATION AND EVALUATION:

Two types of WAFs of Whole Light Alkylate Product were evaluated to demonstrate equilibrium and maintenance of test material. A WAF prepared with freshwater was evaluated at 0,1,3,6,24,36,48,60 and 72 hours after preparation while a WAF prepared with saltwater was evaluated at 0,1,3,6,12,24,36 and 48 hours after preparation. The WAFs were generated following modification of the procedure used by Anderson, et al (1974, Marine Biol., 27: 75-88). Two WAFs were prepared, using each water type, containing 50 ppm of Whole Light Alkylate Product. One WAF of each water type was prepared in a bottle filled to the neck to minimize headspace ("XXX1X" sample designation, e.g, sample "3FW2B" is a 3-hour, freshwater, type 1 WAF, the second of duplicate samples collected), while the second WAF of each water type was prepared in a bottle filled to the shoulder to maximize product-water contact ("XXX2X" sample designation). Duplicate samples were collected from each bottle (except for time zero "XXX2X" series) at the specified time periods, with one sample analyzed using the methodology determined from the in-house validation and the other sample acting as a backup. All samples were collected in 40 ml glass vials with no headspace. The concentration in each flask was quantified to evaluate the consistency of the WAF with time, water type and stirring procedure.

GOOD LABORATORY PRACTICES:

This study was conducted according to the EPA Good Laboratory Practice Standards outlined in 40 CFR Part 160, Federal Register Vol. 54, No.158, 8/17/89.

Test Substance(s) Characterization - The methods of synthesis, fabrication, and/or derivation of the test materials is the responsibility of the sponsor. In addition, the stability, identity, strength, purity and composition of other characteristics which identify the test materials are the responsibility of the sponsor. The test article data are located at the sponsor's facility.

Chemical purity and stability data for reference and control standards purchased commercially, with the exception of 2,3,3-trimethylpentane and 1-methyl-ethylcyclopentane, were provided by the suppliers (Supelco, Sigma). The latter two compounds were assayed for purity at Stonybrook Laboratories Inc. These data and those provided by the suppliers are archived with the raw data.

RECORDS MAINTAINED:

The study file contains but is not limited to the following records or verified copies of:

- Notice of Intent to Initiate Study
- Request for Testing
- Sponsor Protocol Amendment Approval Memo
- Study Protocol and Amendments
- Technical Personnel Records
- Reagents and Equipment Inventory
- Chemical Repository Unit (CRU) Dispensing Records
- Study Notebook Records

RESULTS & DISCUSSION

METHOD EVALUATION/VALIDATION:

The use of the PT/GC technique for the analysis of whole light alkylate product WAF was based on a review of the test article composition and the anticipated composition of the WAF. The use of PT/GC runs throughout the EPA analytical methods series for drinking water (500), municipal/industrial effluent water (600) and wastewater (8000). The method has been tentatively validated for the analysis of gasoline range organics (GRO) in the last year and drafts of the method were made available by the Office of Solid Waste (OSW) prior to the expected promulgation in late 1994.

Six alkanes and one cycloalkane were selected (representing 68% of the components of the test material) for the in-house evaluation/validation. Hexane was chosen as the surrogate. The EPA procedure for the evaluation of method performance is an appropriate standard by which to assess in-house method validation. Determination of the method detection limit (MDL), limit of detection (LOD) and limit of quantitation (LOQ) provide an excellent measure of the sensitivity and precision of the procedure. MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The LOD is the lowest concentration that can be determined statistically differently from the blank. LOD is numerically defined as three times the standard deviation from replicate measurements of standard. LOQ is the level above which quantitative results may be obtained and is numerically defined as ten times the standard deviation from replicate measurements of standards. The LOD, LOQ and MDL were determined from replicate measurements of the analytes and surrogate in water at 5 PPB. In general, the per component MDL was slightly below 5 PPB. The LOD, LOQ and MDL for each of the compounds is reported in table I.

WAF GENERATION AND EVALUATION:

Two types of WAF were generated to evaluate the affect of mixing and headspace on final WAF concentration. The concentration of test article components was significantly higher (factor of 2) in the "minimal headspace" type WAF as compared to the "maximum phase interface" type WAF. Table II reports the time course of WAF concentration for the individual and summed seven analytes monitored for both freshwater (through 72 hours) and saltwater (through 48 hours). The surrogate recoveries, which were essentially quantitatve, are also reported for each WAF sample analyzed.

WAF concentration of test material peaked at approximately 12 hours in saltwater (0.9 PPM) and 24 hours in freshwater (1.6 PPM) using the "minimal headspace"WAF generation procedure. This can be seen more clearly in Figure 1 where the "Total" column data in table II for freshwater and saltwater WAF concentrations are plotted vs time of sampling in a histogram format. Figure 2 plots the individual component concentrations for freshwater and saltwater WAF vs sampling time and shows that the relative concentration of the individual test article WAF components is largely maintained over the mixing period. Figures 3 and 4 compare the 24 hour WAF concentration of the test article components with the actual concentration of the components in the test article. These experimentally observed results can be predicted with a reasonable degree of accuracy if the water solubility or octanol/water partition coefficients of the components are taken into consideration.

Table 1

Summary Sheet for LOD, LOQ and MDL Determinations for Whole Light Alkylate Product WAF Components and Surrogate

Peak#	Compound	Rt. (min.)	Area count						
			Run1	Run2	Run3	Run4	Run5	Run6	Run7
1	2,3-dimethylbutane	8.065	37422	31896	36712	34842	30650	20817	46749
2	hexane (surr)	8.850	35696	30159	34788	34045	28001	19625	46398
3	2,4-dimethylpentane	9.600	41098	34678	40270	37909	33107	22357	52058
4	2,2,4-trimethylpentane	11.420	43538	36274	41736	41538	36182	25682	54862
5	2,5-dimethylhexane	12.750	40754	34303	39308	40101	34222	24153	51361
6	2,3,4-trimethylpentane	13.480	41512	35834	41050	42265	37296	25760	53336
7	2,3,3-dimethylpentane	13.680	41454	36507	41116	42461	38096	35948	41716
8	1-methyl-1-ethylcyclopentane	15.115	46836	42044	46061	49500	44715	45595	47700

Peak#	Compound	Rt. (min.)	FFSCL ODLOQ R1	FFSCL ODLO QR2	FFSCL ODLO QR3	FFSCL ODLOQ R4	FFSCL ODLOQ R5	FFSCL ODLO QR6	FFSCL ODLO CR7	RF(ave)	Std. Dev.	%RSD	Std. Dev. (ppb)	LOD (ppb)	LOQ (ppb)	t value*	MDL (ppb) =Std. Dev. x t
			Response factor=Area count/5 (ng)														
1	2,3-dimethylbutane	8.065	7484.4	6379.2	7342.4	6968.4	6130.0	4163.4	9349.8	6831.1	1573.9	23.0	1.2	3.5	12	3.71	4.3
2	hexane (surr)	8.850	7139.2	6031.8	6957.6	6809.0	5600.2	3925.0	9279.6	6534.6	1637.6	25.1	1.3	3.8	13	3.71	4.6
3	2,4-dimethylpentane	9.600	8219.6	6935.6	8054.0	7581.8	6621.4	4471.4	10411.6	7479.8	1805.8	24.2	1.2	3.6	12	3.71	4.5
4	2,2,4-trimethylpentane	11.420	8711.6	7254.8	8347.2	8307.6	7236.4	5136.4	10972.4	7995.2	1774.6	22.2	1.1	3.3	11	3.71	4.1
5	2,5-dimethylhexane	12.750	8150.8	6860.6	7861.6	8020.2	6844.4	4830.6	10272.2	7548.6	1656.2	21.9	1.1	3.3	11	3.71	4.1
6	2,3,4-trimethylpentane	13.480	8302.4	7166.8	8210.0	8453.0	7459.2	5152.0	10667.2	7915.8	1658.5	21.0	1.0	3.1	10	3.71	3.9
7	2,3,3-dimethylpentane	13.680	8290.8	7301.4	8223.2	8492.2	7619.2	7189.6	8343.2	7922.8	538.9	6.80	0.34	1.0	3.4	3.71	1.3
8	1-methyl-1-ethylcyclopentane	15.115	9371.2	8408.8	9212.2	9900.0	8943.0	9119.0	9540.0	9213.5	471.2	5.11	0.26	0.77	2.6	3.71	0.95

* t value at 99% confidence interval

Table II
Continued

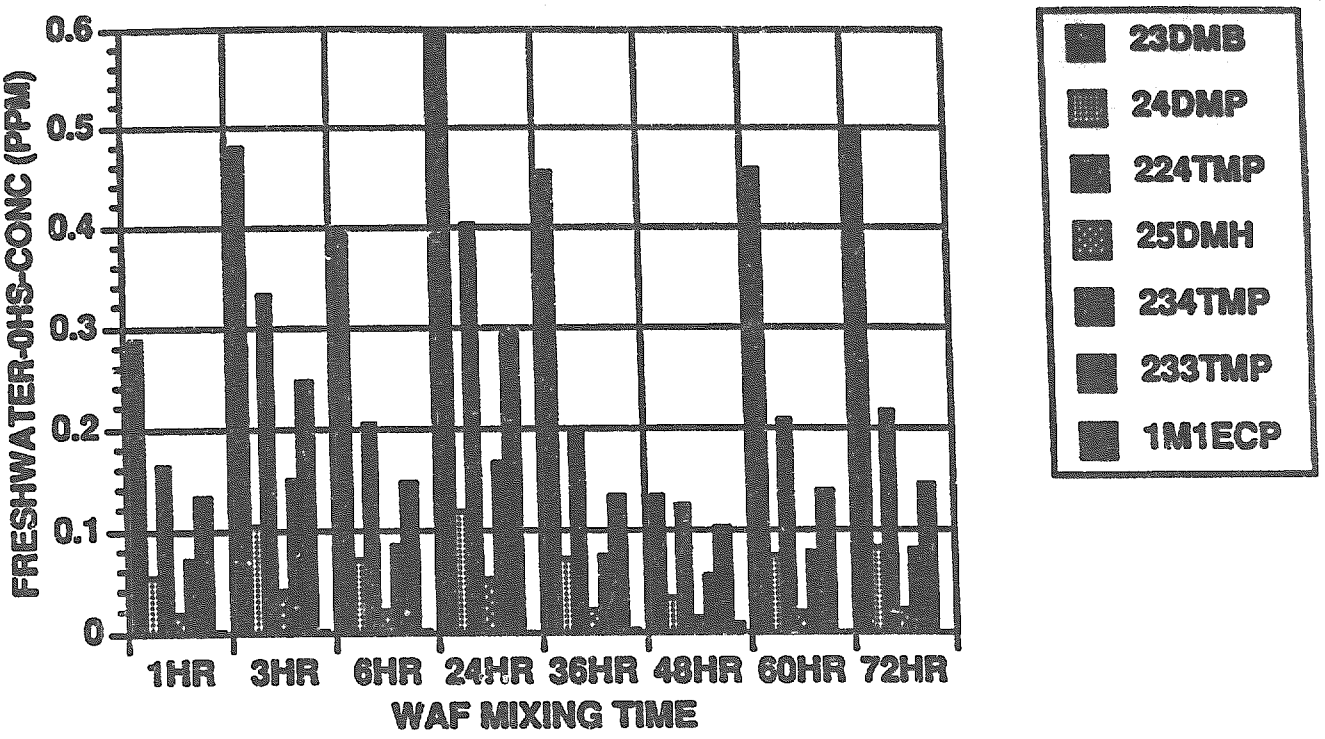
		2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total	hexane (surr) recovery (%)
	RF(ave)	*7524.2*	*8025.4*	*8569.9*	*8495.5*	*8756.4*	*8818.6*	*9204.0*		*7378.6*
Data file	DF*									
36FW1A	20	0.455	0.072	0.197	0.022	0.073	0.133	0.002	0.953	111
36SW1A	10	0.372	0.056	0.145	0.016	0.055	0.101	0.002	0.747	114
36FW2A	20	0.206	0.041	0.128	0.014	0.055	0.103	0.000	0.547	109
36SW2A	10	0.174	0.036	0.117	0.014	0.052	0.100	0.000	0.492	102
48FW1A	20	0.132	0.031	0.125	0.016	0.054	0.102	0.006	0.465	110
48SW1A	10	0.327	0.049	0.134	0.013	0.050	0.099	0.000	0.672	109
48FW2A	20	0.331	0.062	0.186	0.019	0.074	0.140	0.000	0.812	81
48SW2A	10	0.166	0.033	0.100	0.012	0.044	0.084	0.000	0.438	114
60FW1A	20	0.456	0.074	0.206	0.021	0.076	0.138	0.000	0.972	102
60FW2A	20	0.242	0.055	0.184	0.024	0.082	0.146	0.000	0.733	109
72FW1A	20	0.500	0.081	0.216	0.023	0.079	0.143	0.000	1.042	109
72FW2A	20	0.222	0.046	0.142	0.016	0.059	0.110	0.000	0.595	101

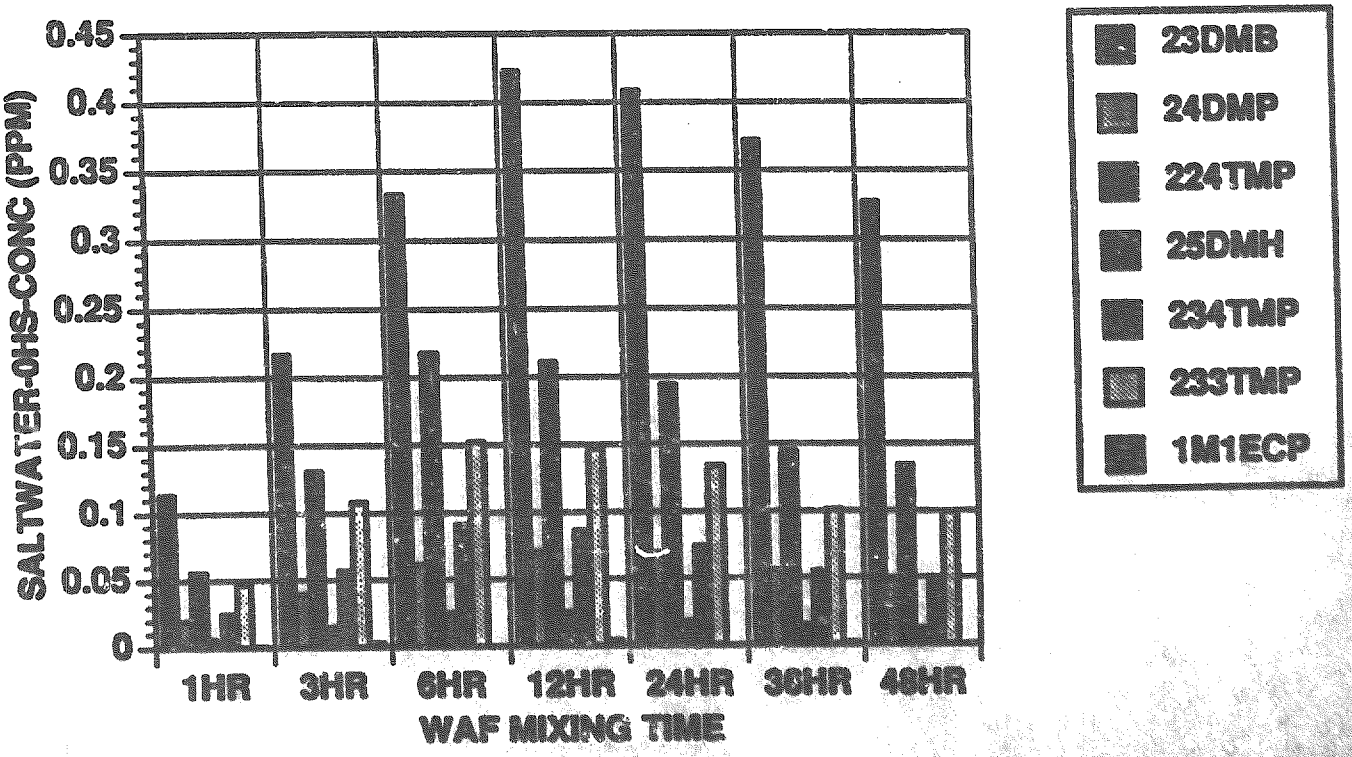
*dilution factor

Data file format - e.g., 36FW1A = 36 hour collection time, freshwater, type "1" WAF (see experimental section), "A", first of two (duplicate) samples collected at the indicated time point.

Figure 2

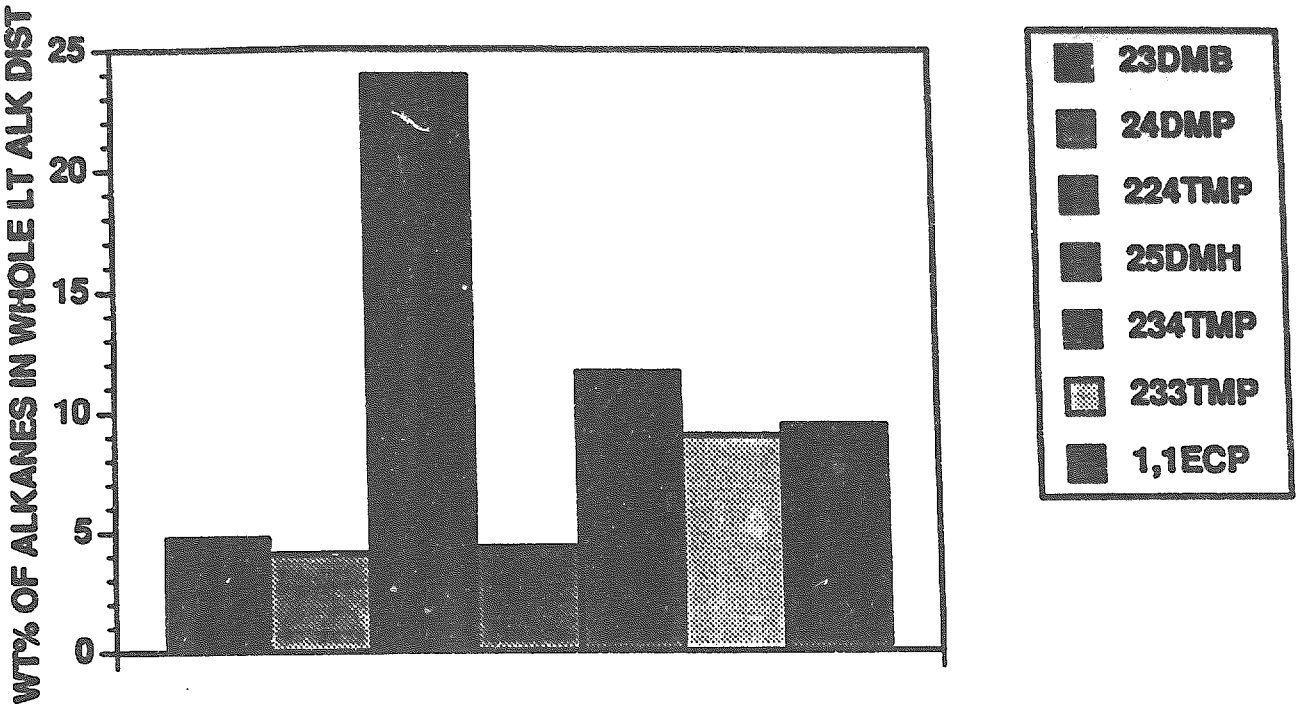
Individual Monitored Component Concentrations in Whole Light Alkylate Product Freshwater and Saltwater WAFs over 48-72 Hours

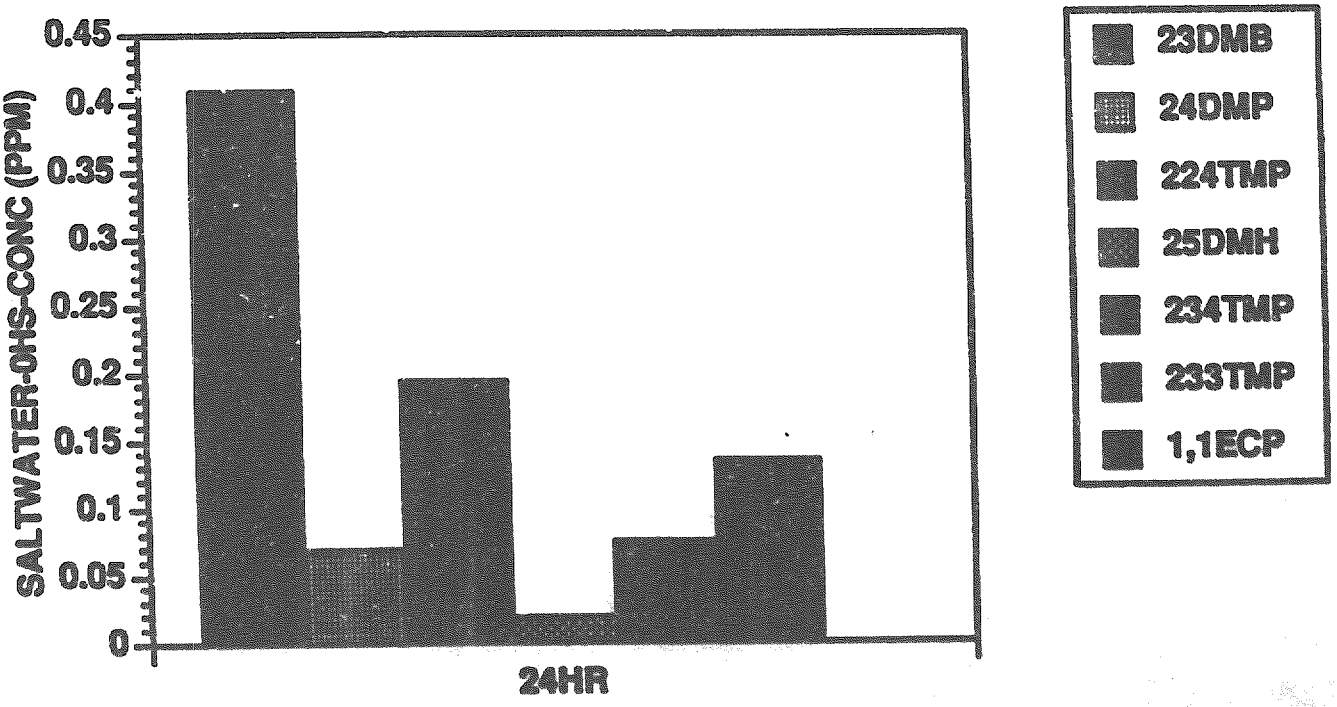




Comparison of Whole Light Alkylate Product Alkane Concentrations In The Neat Material With Their 24 Hour WAF Concentration (Saltwater)

65969 10/26/94 DATA





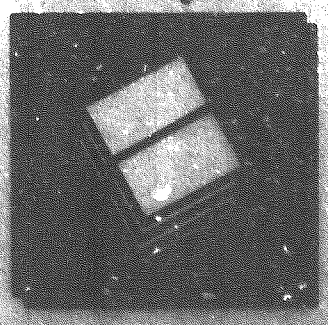
Stonybrook
Laboratories Inc.

Static-Renewal 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to Mysid Shrimp

Stonybrook Laboratories Inc.
Princeton, NJ

Final Report

Study Number 65910



STONYBROOK LABORATORIES INC.
REPORT RELEASE

TO STUDY DIRECTOR/LIAISON: C.A. Schreiner
STUDY NUMBER: 65910
CRU NUMBER: 94194
SAMPLE NAME: Whole Light Alkylate Product
STUDY TITLE: Static-Renewal 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to Mysid Shrimp
REQUESTER: Petroleum Product Stewardship Council

RESULTS: LC_{50} 13.8 ppm for Whole Light Alkylate Product (nominal)
LC_{50} 272 ppb for Whole Light Alkylate Product (measured)

A static-renewal 96-hour toxicity study was conducted June 13-17, 1995 to determine the acute toxicity of Whole Light Alkylate Product to mysid shrimp, a representative salt water invertebrate species. Test mysids were exposed to individual water accommodated fractions (WAFs) of the poorly water-soluble test material at nominal concentrations of 0.6 ppm, 2.5 ppm, 9.2 ppm, 18 ppm, and 49 ppm (w/v, based on density). Nominal test concentrations are based on the loading rate, or amount of test material added to make each WAF. Test solutions were renewed at 24 hour intervals during conduct of the study. Water quality parameters of pH, temperature, salinity, and dissolved oxygen (D.O.) were measured throughout the study.

Samples of the exposure solutions were collected daily and quantitatively analyzed using purge-and-trap/gas chromatography (GC). The concentrations were quantitated using standard Whole Light Alkylate Product component standards. Measured test concentrations are based on the total concentration of all analytes. Test material retention from the static-renewal procedure ranged from 31.0-59.0%. Daily initial measured concentrations indicated consistent exposure of the test mysids to Whole Light Alkylate Product throughout the study.

The toxicity of the test material was evaluated, for nominal and measured concentrations, on the basis of LC_{50} determinations at 24, 48, 72, and 96 hours. The term LC_{50} used in this report refers to the concentration causing 50% mortality after a specified exposure period. The computer-estimated 96-hour LC_{50} for Whole Light Alkylate Product to mysid shrimp under static-renewal test conditions was 13.8 ppm based on nominal exposure concentrations, and 272 ppb based on mean measured exposure concentrations. The 96-hour no observed effect concentration (NOEC), based on nominal concentrations, was 9.2 ppm, since exposure to concentrations of 18 ppm and greater resulted in significant mortality. The 96-hour no observed effect concentration (NOEC), based on measured concentrations, was 218 ppb, since exposure to concentrations of 315 ppb and greater resulted in significant mortality.

Approvals:

J.F. Barbieri/MTB 12/1/95
Study Director/Date
J.F. Barbieri

M.T. BenKinney 12/1/95
Supervisor/Date
M.T. BenKinney

C.R. Mackerer 12/1/95
President/Date
C.R. Mackerer

Distribution: Study Director, Liaison, Archives (Original)

STATIC-RENEWAL 96-HOUR ACUTE TOXICITY STUDY OF THE WATER ACCOMMODATED FRACTION (WAF) OF WHOLE LIGHT ALKYLATE PRODUCT TO MYSID SHRIMP

STUDY No.: 65910

MATERIAL TESTED:	**Whole Light Alkylate Product**
CRU SAMPLE No.:	**94194**
REQUESTER:	**Petroleum Product Stewardship Council c/o Synthetic Organic Chemical Manufacturing Association 1100 NY Ave., NW, Suite 1090 Washington, D.C. 20005**
STUDY PERFORMED BY:	**Stonybrook Laboratories Inc. 311 Pennington-Rocky Hill Road Pennington, N.J. 08534**
STUDY INITIATION DATE:	**July 22, 1994**
EXPERIMENTAL START DATE:	**November 17, 1994**
EXPERIMENTAL TERMINATION DATE:	**June 22, 1995**

Compliance Statement

Study No. 65910

This study was conducted according to the USEPA Toxic Substances Control; Good Laboratory Practice Standards. 40 CFR Part 792, except as noted below; the final report fully and accurately reflects the raw data generated in the study.

Exceptions to GLPs:

1. The test material, Whole Light Alkylate Product, was not characterized and stability analysis was not performed at this facility.

2. Some data entries were made late. These late entries were indicated as such.

3. Some equipment logs were not up to date at the time of the study.

4. Some documentation was missing from the study record, specifically some Artemia (food) preparation records and some acclimation records.

L. F. Barbin MBK 12/1/95

Study Director Date

STONYBROOK LABORATORIES INC.

QUALITY ASSURANCE STATEMENT

Study Number: 65910

Title of Study: Static-Renewal 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to Mysid Shrimp

Listed below are the dates that this study was reviewed by the Quality Assurance Unit and the dates that the findings were reviewed by the Study Director and Management.

DATE(S) OF QA REVIEW	PHASE OF STUDY	DATE(S) REVIEWED BY STUDY DIRECTOR	DATE(S) REVIEWED BY MANAGEMENT
11/18/94	PROTOCOL REVIEW	1/9/95	1/12/95
12/15/94	IN-PROCESS INSPECTION	2/24/95	2/27/95
12/20/94	IN-PROCESS INSPECTION	2/19/95	2/25/95
3/22/95	REPORT AUDIT	3/30/95	5/20/95
8/16/95	FINAL REPORT AUDIT	8/18/95	8/23/95

Lisa Trick
Manager, Quality Assurance

12/1/95
Date

PRINCIPAL INVESTIGATOR

STUDY DIRECTOR 12/1/95

*no longer with company
MTB 12/1/95

DISTRIBUTION:

Liaison:	C.A. Schreiner, Ph.D.
Principal Investigator:	A.L. Wagstaff, B.A.
Study Director:	J.F. Barbieri, B.S.
Supervisor:	M.T. BenKinney, M.S.
President, Stonybrook Laboratories Inc.:	C.R. Mackerer, Ph.D.
Archives	

Additional Personnel Involved in the Study

N.L. Afonina : Laboratory Technician

A.J. Canale : Laboratory Technician

C.W. Chuang : Study Chemist

A.L. Crawford : Culturist

J.S. Gross : Laboratory Technician

A.L. McClurg : Laboratory Technician

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SUMMARY:

A static-renewal 96-hour toxicity study was conducted June 13-17, 1995 to determine the acute toxicity of Whole Light Alkylate Product to mysid shrimp, a representative salt water invertebrate species. Test mysids were exposed to individual water accommodated fractions (WAFs) of the poorly water-soluble test material at nominal concentrations of 0.6 ppm, 2.5 ppm, 9.2 ppm, 18 ppm, and 49 ppm (w/v, based on density). Nominal test concentrations are based on the loading rate, or amount of test material added to make each WAF. Test solutions were renewed at 24 hour intervals during conduct of the study. Water quality parameters of pH, temperature, salinity, and dissolved oxygen (D.O.) were measured throughout the study.

Samples of the exposure solutions were collected daily and quantitatively analyzed using purge-and-trap/gas chromatography (GC). The concentrations were quantitated by GC using standard Whole Light Alkylate Product component standards. Measured test concentrations are based on the total concentration of all analytes. Test material retention from the static-renewal procedure ranged from 31.0-59.0%. Daily initial measured concentrations indicated consistent exposure of the test mysids to Whole Light Alkylate Product throughout the study.

The toxicity of the test material was evaluated, for nominal and measured concentrations, on the basis of LC_{50} determinations at 24, 48, 72, and 96 hours. The term LC_{50} used in this report refers to the concentration causing 50% mortality after a specified exposure period. The computer-estimated 96-hour LC_{50} for Whole Light Alkylate Product to mysid shrimp under static-renewal test conditions was 13.8 ppm based on nominal exposure concentrations, and 272 ppb based on mean measured exposure concentrations. The no observed effect concentration (NOEC), based on nominal concentrations, was 9.2 ppm, since exposure to concentrations of 18 ppm and greater resulted in significant mortality. The no observed effect concentration (NOEC), based on measured concentrations, was 218 ppb, since exposure to concentrations of 315 ppb and greater resulted in significant mortality.

INTRODUCTION:

The objective of this study was to determine the acute toxicity of Whole Light Alkylate Product to aquatic organisms by evaluating its effect on mysid shrimp (*Mysidopsis bahia*), a representative salt water invertebrate species. Mysid shrimp were selected since they are a salt water test species recommended in U.S. EPA (1) regulations. Static-renewal testing of the water accommodated fraction (WAF) was chosen as the most appropriate study design, due to the volatile nature of the test material. Under WAF exposure conditions, toxic effects from the soluble components of the test material are evaluated.

The analytical standards chosen to evaluate the WAF of Whole Light Alkylate Product were selected as representative of the alkane and cycloalkane constituents which account for 68% of the test material. These constituents were expected to be found in the highest concentrations in the WAF and account for most, if not all, of the toxicity measured during the study.

In acute toxicity tests, the most commonly used adverse effect criterion is death of the organism. Mortality data collected during the study are used to calculate an LC_{50} (concentration lethal to 50% of the test population after a specific time period which is typically 96 hours).

METHODS AND MATERIALS:

Test Organisms:

The mysid shrimp (*Mysidopsis bahia*) used in the study were purchased from Aquatic Indicators, St. Augustine, FL. The mysids were held in a holding chamber which initially were filled with water from the shipping container. Approximately 50% of the shipping water was replaced upon arrival with Mobil Technical Center (MTC) well water (Table 1) that had been salinity adjusted with Forty Fathoms synthetic seawater mix. Mysids were held for a few hours prior to testing following acceptable practices (2,3,4). Mysid shrimp were fed newly-hatched Artemia sp. nauplii (24±6 hours) *ad libitum* upon arrival and daily during the study. Continuous aeration was provided during the holding period to keep the Artemia in suspension (which facilitates feeding) and to help maintain dissolved oxygen levels. The mysids used in the study were 5 days old by study initiation. Mysids were individually transferred into 20 mL plastic cups (2 mysids/cup) of test water. Since mysids cannot be individually identified, the organisms in each cup were arbitrarily added to the test containers.

Test System:

The Whole Light Alkylate Product static-renewal toxicity study was conducted in labeled pint glass jars, sealed with teflon lined screw caps. Test jar labeling included the study number, CRU number, test date, concentration group number, replicate letter, and species designation. Test jars contained 473 ml of test solution, allowing no headspace. The water source for the study was MTC well water adjusted to a salinity of 20±2 ppt. The test exposure chambers were held in an incubator maintained at 20 ± 1 °C. The photoperiod during testing was 16-hr light/8-hr dark (fluorescent lighting).

The mysid shrimp were exposed to individual WAF solutions of Whole Light Alkylate Product. Generation of the WAF solutions was produced following a modification of the procedure used by Anderson, et al., 1974 (5). Six individual WAF bottles (2 liter) were set up. A stir bar and 2.28 liters of test water were placed into each bottle. A 2 liter bottle filled to the neck (instead of the normal shoulder height) can hold 2.28 liters. The bottles were filled to neck height to minimize volatility. A measured amount of Whole Light Alkylate Product (nominal concentration), calculated for each exposure concentration, was added to each bottle. All bottles were capped tightly with a teflon lined stopper and parafilm. All aspirator bottles were covered completely with aluminum foil. The stirring speeds of the bottles were adjusted to produce less than a 25% vortex. The solutions stirred for approximately 12 hours, and then were allowed to settle for approximately 45 minutes, except for the range finding study which allowed a 1 hour and 40 minute settling period at test initiation. After the stirring/settling period, the aqueous phase (WAF) was collected through the aspirator spout. Two 473 ml replicates were prepared from each individual WAF. A sample was also collected to take initial water quality measurements. The solution in each test container was renewed daily during the study. The renewal concentrations were produced in the same manner as the initial concentrations. The test mysids remained in the test container during the renewal process.

Test Material:

The test material, Whole Light Alkylate Product, was dispensed by Stonybrook Laboratory's Chemical Repository Unit (CRU) from a homogeneous sample obtained from the sponsor. As reported in the Product Physical and Chemical Data (PPCD) sheet, Whole Light Alkylate Product (CRU No. 94194) consists entirely of Light Alkylate Naphtha. It was received as a liquid. The stability, identity, strength, purity, and composition or other

characteristics which identify the test material are the responsibility of the sponsor. The concentrations used in this study were prepared by pipetting known quantities into each WAF bottle on a weight to volume basis, based on the density (0.7 g/ml) of the test material. Following a stirring and settling period, the aqueous phase of each solution was used for its corresponding exposure concentration.

Test Procedure-Biological:

A range finding study which was not protocol driven was performed on October 19-21, 1994. The results of this analysis were not used in the study.

The range finding test discussed in the protocol was run November 17-19, 1994. This study was performed using static renewal procedures with sealed test chambers allowing as little headspace as possible. Test mysids were exposed to a control and concentrations of 0.92 ppm, 9.2 ppm, and 92 ppm. At test termination, no mortality was observed in the control, with slight mortality (3 mysids, 15%) in the 0.92 ppm concentration. Also at 48 hours, nearly total mortality (18 mysids, 90%) was found in the 9.2 ppm concentration, with total mortality in the 92 ppm concentration. Based on these results, a dose range of 0.3-9.2 ppm was determined for the definitive study.

An initial 96-hour definitive study was conducted December 12-16, 1994, consisting of a control and test concentrations of 0.3 ppm, 0.6 ppm, 1.2 ppm, 2.5 ppm, and 9.2 ppm. This study was conducted using a closed container, static-renewal test procedure, with daily replacement of solution in each test chamber. At test termination, no mortality was observed in the 0.3 ppm concentration, with insignificant mortality (1 mysid, 5%) in the control, 0.6 ppm, and 1.2 ppm concentrations. Also at 96 hours, partial mortality was observed in the 2.5 ppm (5 mysids, 25%) and 9.2 ppm (9 mysids, 45%) concentrations. Since no exposure concentration produced greater than 50% mortality, the 96-hour LC_{50} was >9.2 ppm, the highest concentration. A second definitive run was conducted, with a higher dose range, to determined the actual LC_{50}.

A second definitive toxicity study was conducted December 19-23, 1994. This study was conducted using a static-renewal test procedure, with daily replacement of solution in each test chamber. Unfortunately during this definitive study run, two sets of chemical analysis samples were, inadvertently, not taken. Due to the relative importance of these two sets of chemical analyses to the determination of the measured toxicity results, the study was rerun.

The definitive toxicity study documented in this report was conducted June 13-17, 1995. This study was conducted using a static-renewal test procedure, with daily replacement of solution in each test chamber. All concentrations were run in duplicate in pint glass jars containing 473 ml of solution, with no headspace. Mysid shrimp were arbitrarily added, two at a time, until each replicate contained 10 mysids, within one hour of initial WAF solution preparation. The test chambers were held in an incubator (20 ± 1 °C), and sealed with teflon lined screw caps to minimize volatilization. Exposure concentrations with surviving mysids were renewed at each 24-hour interval during conduct of the study by siphoning the final solutions out of each test chamber, leaving only enough volume so that the organisms were not distressed. A sample of each final solution was retained for water quality analysis during the renewal. The newly prepared solution (approximately 473 ml) was then carefully poured into each test chamber to complete the renewal.

The mysids were exposed to a control and five nominal concentrations (0.6 ppm, 2.5 ppm, 9.2 ppm, 18 ppm, and 49 ppm) of Whole Light Alkylate Product. The control consisted of the same dilution water, test conditions, and test organisms with no added test material. The mysids in each test chamber were observed daily for survival at 1, 3, 6, and 24 hour intervals. Observations at 1, 3, and 6 hours were made with the jar lids remaining on, to minimize volatilization. The 24, 48, 72 and 96 hour observations were made with the lids

removed, during renewal or at termination. At each observation period, the mysids remaining alive in all exposure concentrations were counted. Live animals were counted in all chambers to account for mortality, cannibalism, and missing mysids. Dead individuals were removed from the jars at each renewal period. At each observation period, mysids were also observed for behavioral abnormalities such as being hyperactive, lethargic, movement only on prodding, and erratic swimming.

Test Procedure-Water Quality:

Water quality parameters of dissolved oxygen (D.O.), pH, salinity, and temperature were measured at study initiation and daily in a portion of the freshly-prepared initial sample containing no test organisms. These water quality parameters were also taken daily in final replicate samples. Water quality was performed only on final samples from test chambers that contained some living organisms at the previous observation period, and in initial samples from chambers with some living organisms present. Dissolved oxygen was measured with a YSI Model 57 D.O. Meter with a Model 5739 D.O. probe. The pH was measured with an Orion Model 520A Digital pH/mV Meter with an Orion Model 81-02 Combination pH Electrode. Salinity was measured with a Spartan Model A366ATC Salinity Hand Refractometer. Temperature was measured with a hand-held thermometer, with a stainless steel thermocouple.

Test Procedure-Chemical:

Chemical analysis was performed on 40 ml samples of the initial WAF solutions of the control and exposure concentrations at test initiation, 24, 48, and 72 hours and on final samples of the control and exposure concentrations at 24, 48, 72, and 96 hours. Chemical analysis was performed only on final samples from test chambers that contained some living organisms at the previous observation period, and in initial samples from chambers with some living organisms present. Final samples were a composite of the two replicates. The samples were collected in 40 ml jars with septum caps with no head space, and transferred to the Analytical Chemistry group for analysis. Chemical analysis was performed within 14 days of sample collection. The concentration of Whole Light Alkylate Product in each sample (measured concentration) was determined by using purge-and-trap and a gas chromatograph equipped with a flame ionization detector (GC-FID) following the methods developed in the methods validation study (Study No. 65969) (Appendix 2). The following components of Whole Light Alkylate Product were quantified: 2,3-dimethyl butane, 2,4-dimethyl pentane, 2,2,4-trimethyl pentane, 2,5-dimethyl hexane, 2,3,4-trimethyl pentane, 2,3,3-trimethyl pentane, and 1-methyl-1-ethyl-cyclopentane. Based on the method validation study, these components represent 68% of the composition of Whole Light Alkylate Product. All chemical analyses (Appendix 1) were performed by C.W. Chuang of the Analytical Chemistry Group.

Statistical Analysis:

Daily LC_{50} values were calculated on the basis of mortality data and nominal/measured dose levels. Statistical analysis of the data was calculated by a computer software LC_{50} program developed by Stephan et al. (6). This program statistically calculates the LC_{50} using binomial probability analysis, moving average angle analysis, and probit analysis. The LC_{50} was also calculated using the Spearman-Karber method (7,8). These different methods of analyzing the data are used since no one method of analysis is appropriate for all possible sets of data that may be obtained (9). The no observed effect concentration values were calculated using Fisher's exact test (9). The method selected for analysis of the data present in this report was determined by the characteristics of the data base.

Daily measured dose levels, for each concentration, were a cumulative total of all sample values evaluated between the initial sample and the final sample, inclusive, for that

time period. Measured dose levels were the cumulative total of all measured test material components, for each concentration. In cases where the measured component levels were below that component's detection limit, a zero value was used in the calculations. For the 96 hour time period (all samples), a standard deviation was also calculated. The average measured levels for each time period were used along with corresponding survival data to produce measured LC_{50} and NOEC values. Also for each concentration, all initial sample values were averaged, and all final sample values were averaged. The percent difference between initial and final averages was used to calculate the average percent retention at each exposure period.

Data Storage:

The study was conducted according to the EPA Good Laboratory Practice Standards (40 CFR Part 792) (10). Raw data (Appendix 3) and the original final report are maintained in the Archives of Stonybrook Laboratories Inc. located in Pennington, New Jersey.

RESULTS:

The LC_{50} values for the 96-hour static-renewal toxicity study of Whole Light Alkylate Product to mysid shrimp (*Mysidopsis bahia*) are summarized in Table 2. Based on nominal exposure concentrations, the 24, 48, 72, and 96 hour LC_{50} values and 95% confidence intervals were 52.7 ppm (39-108 ppm), 26.6 ppm (18-49 ppm), 16.0 ppm (12.9-20.5 ppm), and 13.8 ppm (11.1-17.4 ppm), respectively. Based on daily measured exposure concentrations, the 24, 48, 72, and 96 hour LC_{50} values and 95% confidence intervals were 415 ppb (319-749 ppb), 349 ppb (260-552 ppb), 275 ppb (239-321 ppb), and 272 ppb (241-310 ppb), respectively. The 24, 72, and 96 hour LC_{50} values were determined by probit analysis. The 48 hour LC_{50} value were determined by binomial probability analysis. Cumulative survival data for this study are presented in Table 3. Behavioral effects are presented in Table 4.

Water quality parameters of pH, dissolved oxygen, salinity, and temperature were performed only for initial samples from chambers with some living organisms present, and for final samples from test chambers that contained some living organisms at the previous observation period. Mean values and the range for each test chamber are summarized in Tables 5 and 6.

The measured concentrations of Whole Light Alkylate Product in the test chambers were determined by purge-and-trap/gas chromatography (Appendix 1). The concentrations listed in this appendix are based on the coding system identified in the raw data where the first character represents the test concentration group as listed in the protocol; the second character represents either an initial (I) or a final (F) sample; and the third and fourth characters represent the hour of the sampling period. The measured exposure concentrations and calculated averages of the samples collected during the study and the percent retention for average initial and final samples collected during the study are summarized in Tables 7 and 8. The chemical analysis techniques used in this study were developed during the Methods Validations study (Study 65969). A copy of this study is provided in Appendix 2.

DISCUSSION:

The salinity and temperature monitored during the study remained within acceptable limits. The pH values remained consistent among concentrations and dissolved oxygen levels remained above 60% saturation in all doses.

One mysid was identified as missing in the controls during the study. Total mortality was observed in the highest concentration, 49 ppm, by 48 hours. At test termination, no mortality was observed in the 0.6 ppm and 2.5 ppm concentrations. Also at 96 hours, partial mortality was observed in the 9.2 ppm concentration (4 mysids, 20%) and in the 18 ppm concentration (14 mysids, 70%). The 96-hour LC_{50} for Whole Light Alkylate Product to mysid shrimp under static-renewal test conditions was, therefore, 13.8 ppm based on nominal exposure concentrations, and 272 ppb based on mean measured exposure concentrations.

It should be noted that by study termination, extreme behavioral effects were noted in the two highest concentrations with survivors. In the 9.2 ppm concentration, only 3 of the survivors were normal, 6 survivors were lethargic, and the other 7 survivors moved only on prodding. In the 18 ppm concentration, 5 of the survivors moved only on prodding and the other survivor was immobile. All behavioral observations are presented in Table 4.

Samples of the control and exposure concentrations were collected daily and quantitatively analyzed using purge-and-trap/gas chromatography (GC). The concentrations were quantitated using standard Whole Light Alkylate Product component standards. Test material retention from the static-renewal procedure ranged from 31.0 to 59.0%. Daily initial measured concentrations indicated consistent exposure of the test mysids to Whole Light Alkylate Product throughout the study.

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TABLE 1: Characteristics of MTC Well Water (1 Year Average)

Parameter Measured	Concentration
Dissolved Oxygen	4.8 ppm
pH	7.39
Conductivity	442 µmhos
Total Hardness ($CaCO_3$)	206 mg/L
Alkalinity ($CaCO_3$)	135 mg/L
TSS	<7 mg/L
Ammonia (Distillation as N)	<1 mg/L
Phosphorus (Total as P)	<0.05 mg/L
Sulfate	51 mg/L
COD	<7 mg/L
Cyanide	<0.005 mg/L
Antimony	<0.005 mg/L
Arsenic	<0.01 mg/L
Barium	0.15 mg/L
Beryllium	<0.0002 mg/L
Cadmium	<0.0005 mg/L
Chromium	<0.003 mg/L
Copper	0.102 mg/L
Iron	<0.1 mg/L
Lead	<0.0026 mg/L
Magnesium	20.2 mg/L
Manganese	<0.01 mg/L
Mercury	<0.0002 mg/L
Nickel	<0.05 mg/L
Fluoride	0.1 mg/L
Selenium	<0.005 mg/L
Silver	<0.009 mg/L
Zinc	<0.049 mg/L
TOC	<1 mg/L
NO_3-N	<2 mg/L
Thallium	<0.002 mg/L
Phenols	<0.005 mg/L
Lindane	<0.01 µg/L
Methoxychlor	<0.05 µg/L
Endrin	<0.01 µg/L
Toxaphene	<4 µg/L

TABLE 2: Acute Toxicity of Whole Light Alkylate Product to Mysid Shrimp

	LC_{50} (95% Confidence Limits)***			
	24 Hrs*	48 Hrs**	72 Hrs*	96 Hrs*
Nominal	52.7 ppm (39-108 ppm)	26.6 ppm (18-49 ppm)	16.0 ppm (12.9-20.5 ppm)	13.8 ppm (11.1-17.4 ppm)
Measured	415 ppb (319-749 ppb)	349 ppb (260-552 ppb)	275 ppb (239-321 ppb)	272 ppb (241-310 ppb)

* LC_{50} values calculated using Probit Analysis.
** LC_{50} values calculated using Binomial Probability Analysis.
*** 95% Confidence Limits for 24, 72 and 96 hours. The 95% confidence limits presented for 48 hours are not actually confidence limits because the LC_{50} was determined by binomial probability analysis. The limits are statistically sound conservative bounds that are above 95% for the sample size used in this study.

	NOEC*			
	24 Hrs	48 Hrs	72 Hrs	96 Hrs
Nominal	18 ppm	18 ppm	9.2 ppm	9.2 ppm
Measured	155 ppb	260 ppb	191 ppb	218 ppb

* All NOEC values calculated using Fisher's Exact Test.

TABLE 3: Cumulative Survival During the Acute Toxicity Study of Whole Light Alkylate Product to Mysid Shrimp

Exposure Time	Nominal Concentration (ppm)					
	Control	0.6	2.5	9.2	18	49
Day 0:						
1 hrs.	20/20	20/20	20/20	20/20	20/20	20/20
3 hrs.	20/20	20/20	20/20	20/20	20/20	20/20
6 hrs.	20/20	20/20	20/20	20/20	20/20	20/20
24 hrs.	20/20	20/20	20/20	20/20	19/20	11/20
Day 1:						
1 hrs.	20/20	20/20	20/20	20/20	19/20	11/20
3 hrs.	20/20	20/20	20/20	20/20	19/20	11/20
6 hrs.	20/20	20/20	20/20	20/20	19/20	11/20
24 hrs.	19/20	20/20	20/20	18/20	18/20	0/20
Day 2:						
1 hrs.	19/20	20/20	20/20	18/20	18/20	0/20
3 hrs.	19/20	20/20	20/20	18/20	18/20	0/20
6 hrs.	19/20	20/20	20/20	18/20	18/20	0/20
24 hrs.	19/20	20/20	20/20	17/20	9/20	0/20
Day 3:						
1 hrs.	19/20	20/20	20/20	17/20	9/20	0/20
3 hrs.	19/20	20/20	20/20	17/20	9/20	0/20
6 hrs.	19/20	20/20	20/20	17/20	9/20	0/20
24 hrs.	19/20	20/20	20/20	16/20	6/20	0/20

TABLE 4: Behavior Observations During the Acute Toxicity Study of Whole Light Alkylate Product To Mysid Shrimp

Behavior of Survivors	Nominal Concentration (ppm)					
Exposure Time	Control	0.6	2.5	9.2	18	49
Day 0:						
1 hrs.	20N	20N	20N	20N	20N	20L
3 hrs.	20N	20N	20N	20N	20N	13L,2E,5I
6 hrs.	20N	20N	20N	20N	20N	12L,1E,7I
24 hrs.	20N	20N	20N	19N1L	19N1X	2L,9P,9X
Day 1:						
1 hrs.	20N	20N	20N	20N	19N	1L,10I
3 hrs.	20N	20N	20N	20N	15N,2L,2I	11I
6 hrs.	20N	20N	20N	20N	12N,4L,3I	11I
24 hrs.	19N,1M	20N	20N	18N,2Z	9N,5L,4P,1X	11X
Day 2:						
1 hrs.	19N	20N	20N	18N	9N,1L,8I	---
3 hrs.	19N	20N	20N	18N	9N,1L,8I	---
6 hrs.	19N	20N	20N	18N	9N,1L,8I	---
24 hrs.	19N	20N	20N	12N,5L,1X	1N,8L,2Z,7X	---
Day 3:						
1 hrs.	19N	20N	20N	11N,4L,2E	2N,7L	---
3 hrs.	19N	20N	20N	9N,3L,5E	8L,1E	---
6 hrs.	19N	20N	20N	5N,4L,8E	6L,2E,1I	---
24 hrs.	19N	18N, 2P	19N, 1E	3N,6L,7P	5P,1I,3X	---

N - Normal
M - Missing
L - Lethargic
Z - Non-Intact Mysids
X - Dead/Removed
E - Erratic Swimming
H - Hyperactive
P - Movement on Prodding
I - Immobile

TABLE 5: Summary of Initial Water Quality Measurements Taken During the Acute Toxicity Study of Whole Light Alkylate Product to Mysid Shrimp

Test Concentration	Temperature (°C) X*	Range	pH Range
Control	20.5	20.2-20.8	8.37-8.50
0.6 ppm	20.5	19.9-20.8	8.38-8.52
2.5 ppm	20.3	19.5-20.7	8.38-8.51
9.2 ppm	20.1	19.6-20.4	8.39-8.52
18 ppm	20.0	19.2-20.5	8.39-8.52
49 ppm	19.9	19.3-20.4	8.42-8.52

Test Concentration	D.O. (ppm) X*	Range	Salinity (ppt) X*	Range
Control	6.7	6.6-6.8	19	18-19
0.6 ppm	6.7	6.5-6.9	19	18-19
2.5 ppm	6.7	6.5-6.9	19	18-19
9.2 ppm	6.6	6.4-6.8	19	19-20
18 ppm	6.6	6.4-6.8	19	19-20
49 ppm	6.7	6.6-6.7	19	**

* X = Mean Value
** Parameter remained the same throughout the study.

TABLE 6: Summary of Final Water Quality Measurements Taken During the Acute Toxicity Study of Whole Light Alkylate Product to Mysid Shrimp

Test Conc.	Rep.	Temperature (°C) X*	Temperature (°C) Range	pH Range
Control	A	20.3	**	8.35-8.43
Control	B	20.2	20.1-20.2	8.35-8.45
0.6 ppm	A	20.2	20.1-20.3	8.30-8.45
0.6 ppm	B	20.1	20.0-20.1	8.38-8.45
2.5 ppm	A	20.3	20.1-20.5	8.40-8.46
2.5 ppm	B	20.3	20.1-20.4	8.40-8.46
9.2 ppm	A	20.2	20.1-20.3	8.41-8.46
9.2 ppm	B	20.2	20.1-20.3	8.42-8.47
18 ppm	A	20.2	20.1-20.3	8.43-8.47
18 ppm	B	20.2	20.1-20.3	8.43-8.47
49 ppm	A	20.3	20.2-20.3	8.43-8.48
49 ppm	B	20.3	20.2-20.3	8.43-8.48

Test Conc.	Rep.	D.O. (ppm) X*	D.O. (ppm) Range	Salinity (ppt) X*	Salinity (ppt) Range
Control	A	6.2	5.5-6.7	19	19-20
Control	B	6.3	5.8-6.7	19	19-20
0.6 ppm	A	6.2	5.8-6.6	20	19-20
0.6 ppm	B	6.3	6.0-6.6	19	19-20
2.5 ppm	A	6.2	6.1-6.4	19	19-20
2.5 ppm	B	6.1	5.9-6.4	19	19-20
9.2 ppm	A	6.1	6.0-6.4	20	19-20
9.2 ppm	B	6.2	6.0-6.4	20	19-20
18 ppm	A	6.3	6.2-6.5	20	19-20
18 ppm	B	6.5	6.3-6.6	20	19-20
49 ppm	A	6.4	6.3-6.5	20	19-20
49 ppm	B	6.4	6.3-6.5	20	19-20

* X = Mean Value
** Parameter remained the same throughout the study.

TABLE 7: Measured Exposure Concentrations Determined for the Acute Toxicity Study of Whole Light Alkylate Product to Mysid Shrimp

All values in ppm

Sample	0 hr. Initial	24 hr. Final	24 hr. Initial	48 hr. Final	48 hr. Initial	72 hr. Final	72 hr. Initial	96 hr. Final
Control	ND	ND	ND	ND	ND	ND	ND	ND
0.6 ppm	0.013	ND	0.039	0.012	0.028	0.004	0.037	0.019
2.5 ppm	0.055	0.029	0.050	0.032	0.103	0.046	0.105	0.075
9.2 ppm	0.143	0.065	0.330	0.163	0.321	0.125	0.406	0.190
18 ppm	0.212	0.098	0.513	0.215	0.560	0.197	0.500	0.227
49 ppm	0.497	0.283	0.969	0.457	*	*	*	*

* Sample not taken due to complete mortality at 48 hours.

TABLE 8a: Daily Cumulative Averages of the Measured Exposure Concentrations Determined for the Acute Toxicity Study of Whole Light Alkylate Product to Mysid Shrimp

All values in ppm

Sample	24 hr. Avg.	48 hr. Avg.	72 hr. Avg.	All Samples Avg.	All Samples Std. Dev.
Control	ND*	ND	ND	ND	NC**
0.6 ppm	0.006	0.016	0.016	0.019	0.015
2.5 ppm	0.042	0.042	0.052	0.062	0.030
9.2 ppm	0.104	0.175	0.191	0.218	0.120
18 ppm	0.155	0.260	0.299	0.315	0.178
49 ppm	0.390	0.552	0.552	0.552	0.293

TABLE 8b: Initial/Final Averages and Percent Retention of the Measured Exposure Concentrations Determined for the Acute Toxicity Study of Whole Light Alkylate Product to Mysid Shrimp

All values in ppm

Sample	Average of Initial Samples	Average of Final Samples	Average % Retention
Control	ND	ND	NC
0.6 ppm	0.029	0.009	31.0
2.5 ppm	0.078	0.046	59.0
9.2 ppm	0.300	0.136	45.3
18 ppm	0.446	0.184	41.3
49 ppm	0.733	0.370	50.5

* ND = Not Detected
** NC = Not Calculable

APPENDIX 1

STONYBROOK LABORATORIES INC.

To: J. F. Barbieri

Date: July 17, 1995

From: C.W. Chuang

CC: M.T. Benkinney

RE: ANALYSIS OF WHOLE LIGHT ALKYLATE PRODUCT IN WATER ACCOMMODATED FRACTION (WAF)

STUDY NO: 65910

The analysis of whole light alkylate product in WAF was performed following a purge-and-trap/gas chromatography procedure recently validated in-house (Study no. 65969). The results are as follows:

Table 1.1. Concentration of analytes in stock solutions prepared at 0 hour

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1I00	0	ND*	ND	ND	ND	ND	ND	ND	----
2I00	0.6	0.005	ND	0.004	ND	ND	0.004	ND	0.013
3I00	2.5	0.019	ND	0.015	ND	0.007	0.014	ND	0.055
4I00	9.2	0.050	0.011	0.036	ND	0.016	0.030	ND	0.143
5I00	18	0.090	0.017	0.047	ND	0.020	0.038	ND	0.212
6I00	49	0.187	0.037	0.114	0.010	0.051	0.096	0.002	0.497

* ND = not detected at the method detection limit (re: Study no. 65969):

	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane
MDL (ppm)	0.004	0.005	0.004	0.004	0.004	0.001	0.001

Table 1.2. Concentration of analytes of 24-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F24	0	ND	ND	ND	ND	ND	ND	ND	----
2F24	0.6	ND	ND	ND	ND	ND	ND	ND	----
3F24	2.5	0.010	ND	0.007	ND	0.004	0.008	ND	0.029
4F24	9.2	0.026	ND	0.016	ND	0.007	0.016	ND	0.065
5F24	18	0.040	0.007	0.020	ND	0.010	0.019	0.002	0.098
6F24	49	0.106	0.020	0.062	0.005	0.030	0.058	0.002	0.283

Table 2.1. Concentration of analytes in stock solutions prepared at 24 hours

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1I24	0	ND	ND	ND	ND	ND	ND	ND	----
2I24	0.6	0.011	ND	0.011	ND	0.006	0.011	ND	0.039
3I24	2.5	0.017	ND	0.013	ND	0.007	0.013	ND	0.050
4I24	9.2	0.101	0.023	0.083	0.006	0.040	0.077	ND	0.330
5I24	18	0.187	0.037	0.119	0.009	0.054	0.107	ND	0.513
6I24	49	0.363	0.073	0.227	0.022	0.099	0.185	ND	0.969

Table 2.2. Concentration of analytes of 48-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F48	0	ND	ND	ND	ND	ND	ND	ND	----
2F48	0.6	0.004	ND	0.004	ND	ND	0.004	ND	0.012
3F48	2.5	0.011	ND	0.008	ND	0.004	0.009	ND	0.032
4F48	9.2	0.057	0.011	0.039	ND	0.018	0.038	ND	0.163
5F48	18	0.081	0.015	0.049	ND	0.023	0.047	ND	0.215
6F48	49	0.173	0.033	0.105	0.007	0.047	0.092	ND	0.457

Table 3.1. Concentration of analytes in stock solutions prepared at 48 hours

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1I48	0	ND	ND	ND	ND	ND	ND	ND	----
2I48	0.6	0.010	ND	0.007	ND	0.004	0.007	ND	0.028
3I48	2.5	0.030	0.007	0.027	ND	0.013	0.026	ND	0.103
4I48	9.2	0.104	0.023	0.081	0.005	0.037	0.071	ND	0.321
5I48	18	0.186	0.040	0.141	0.012	0.062	0.119	ND	0.560

Table 3.2. Concentration of analytes of 72-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F72	0	ND	ND	ND	ND	ND	ND	ND	----
2F72	0.6	0.004	ND	ND	ND	ND	ND	ND	0.004
3F72	2.5	0.015	ND	0.012	ND	0.006	0.013	ND	0.046
4F72	9.2	0.044	0.009	0.029	ND	0.014	0.029	ND	0.125
5F72	18	0.068	0.014	0.047	ND	0.022	0.046	ND	0.197

Table 4.1. Concentration of analytes in stock solutions prepared at 72 hours

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1T72	0	ND	ND	ND	ND	ND	ND	ND	----
2T72	0.6	0.012	ND	0.010	ND	0.005	0.010	ND	0.037
3T72	2.5	0.031	0.007	0.027	ND	0.013	0.027	ND	0.105
4T72	9.2	0.131	0.028	0.100	0.008	0.047	0.092	ND	0.406
5T72	18	0.176	0.036	0.119	0.009	0.053	0.107	ND	0.500

Table 4.2. Concentration of analytes of 96-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F96	0	ND	ND	ND	ND	ND	ND	ND	----
2F96	0.6	0.007	ND	0.006	ND	ND	0.006	ND	0.019
3F96	2.5	0.022	0.005	0.018	ND	0.010	0.019	0.001	0.075
4F96	9.2	0.064	0.013	0.045	ND	0.022	0.046	ND	0.190
5F96	18	0.083	0.016	0.052	ND	0.024	0.052	ND	0.227

Please call me to discuss the results.

APPENDIX 2

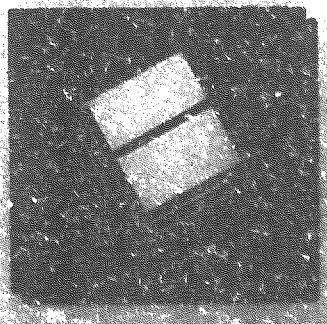
Stonybrook
Laboratories Inc.

Methods Validation for the Analysis of Whole Light Alkylate Product in Water Accommodated Fraction (WAF) Using Purge-and-Trap and GC/FID

Stonybrook Laboratories Inc.
Princeton, NJ

Study Number: 658-99

Final Report



STONYBROOK LABORATORIES INC.

REPORT RELEASE

LIAISON: C.A. SCHREINER

STUDY NUMBER: 65969

CRU NUMBER: 94194

TEST ARTICLE: WHOLE LIGHT ALKYLATE PRODUCT

STUDY TITLE: METHODS VALIDATION FOR THE ANALYSIS OF WHOLE LIGHT ALKYLATE PRODUCT IN WATER ACCOMMODATED FRACTION (WAF) USING PURGE-AND-TRAP AND GC/FID

RESULTS:

The development and validation of a purge-and-trap/gas chromatography (PT/GC) method for the analysis of water acclimated fractions (WAF) of whole light alkylate product and the subsequent determination of optimal WAF equilibration times has been completed. The method was developed and validated using seven C6-C8 alkane and cycloalkane standards which represent 68% of the whole light alkylate product. The sensitivity and precision of the assay were validated at the 5 part-per-billion (PPB) level for each of the seven component standards in water. Using this technique, it was determined that the whole light alkylate product freshwater WAF reached equilibrium in approximately 24 hours at a total WAF concentration (sum of n=7 components) of 1.6 parts-per-million (PPM). The saltwater WAF reached equilibrium in approximately 12 hours at a total concentration (sum of n=7 components) of 0.9 PPM.

T.A. Roy 1/30/95
T.A. Roy Date
Study Director

C.A. Schreiner 1/30/95
C.A. Schreiner Date
Vice-President

C.R. Mackerer 2/1/95
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STUDY NO. 65969

STATEMENT OF COMPLIANCE

The undersigned hereby state that Study No. 65969, Methods Validation for the Analysis of Whole Light Alkylate Product in Water Accommodatated Fraction (WAF) Using Purge-and-Trap and GC/FID, was conducted in compliance with the Good Laboratory Practice Regulations as published in 40 CFR Part 792 Federal Registrar Volume 54-158, 8/17/89 in all aspects with the following exceptions:

The strength, purity and composition or other characteristics to define the test substance was not determined by the testing facility. The methods of synthesis, fabrication, or derivation of the test substance are the responsibility of the sponsor and the data are located at the sponsor's facility.

The purity of purchased reference materials was not determined by the testing facility. It is not known if the purity determination of these chemicals by the supplier were performed under GLPs.

The data acquisition or analysis software on the HP MS DOS operating system used in the study has not been validated in-house.

No bulk inventory usage log was mantained for the test chemicals or analytical standards.

T. A. Roy
Study Director

G. A. Rausina
Study Sponsor

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SUMMARY

The development and validation of a purge-and-trap/gas chromatography (PT/GC) method for the analysis of water accliamated fractions (WAF) of whole light alkylate product and the subsequent determination of optimal WAF equilibration times has been completed. The method was developed and validated using seven C6-C8 alkane and cycloalkane standards which represent 68% of the whole light alkylate product. The sensitivity and precision of the assay were validated at the 5 part-per-billion (PPB) level for each of the seven component standards in water. Using this technique, it was determined that the whole light alkylate product freshwater WAF reached equilibrium in approximately 24 hours at a total WAF concentration (sum of n=7 components) of 1.6 parts-per-million (PPM). The saltwater WAF reached equilibrium in approximately 12 hours at a total concentration (sum of n=7 components) of 0.9 PPM.

EXPERIMENTAL

EXPERIMENTAL DESIGN SUMMARY:

Seven C6-C8 alkanes and cycloalkane, which represent 68% of whole light alkylate product, were selected as the monitored analytes for the in-house method validation. The analyte in methanol solution was spiked into 5 mL deionized water. The aqueous solution were loaded into the purge-and-trap sparger by a Luer Lock syringe. The analytes were then purged out by helium from the aqueous phase to the vapor phase at ambient temperature. The vapor was transferred and consequently trapped in a sorbent tube. After the purge was completed, the sorbent tube was then backflushed and heated. The analytes were swept by helium onto the head of the GC column where the separation and detection took place. The evaluations included measuring each compound's response sensitivity, reproducibility, and purge efficiency. Once the analytical procedure had been verified, a WAF of Whole Light Alkylate Product was generated and evaluated at different time intervals to demonstrate the suitability of the proposed WAF generation procedure.

TEST SUBSTANCES:

ANALYTE NAME	CRU #	LOT #	EXPIRATION	PURITY
2-methylbutane (isopentane)	94570	03859DG	9/99	99%
2,3-dimethylbutane	94565	LA-44304	9/99	99%
2,4-dimethylpentane	94565	LA-44304	9/99	99%
2,5-dimethylhexane	94565	LA-44304	9/99	99%
2,2,4-trimethylpentane	94565	LA-44304	9/99	99%
2,3,4-trimethylpentane	94565	LA-44304	9/99	99%
hexane (surrogate)	110-54-3*	42H06471	1/99	99%
2,3,3-trimethylpentane	94591	244X-5S	10/99	99%
1-methyl-ethylcyclopentane	94590	2360	10/99	99%

*CAS Number

Chemical purity and stability data for reference standards purchased commercially were provided by the suppliers (Supelco, Sigma, Wiley, API Standard Reference Materials). The data provided by the suppliers is archived with the raw data.

APPARATUS AND REAGENTS:

Syringe--5 mL gas-tight glass with Luer Lock.
Micro syringes--10 µL, 25 µL, 50 µL, 100 µL, and 250 µL.
GC vials--Glass with Teflon-lined screw caps.
Volumetric flasks--Varisble volume size with ground-glass stoppers.
Analytical balance--0.0001 g.
Methanol--HPLC grade.

Secondary working standard mixes--Two standard mixes of the eight whole light alkylate component alkanes plus hexane were prepared by mixing their individual stock standard in methanol for a concentration of 100 µg/mL: mix I: isopentane, 2,3, 3-trimethypentane, and 1-methyl-1-ethylcyclopentane and mix II contained the remaining 5 analytes plus the surrogate, hexane.

Calibration standards--Five levels of standards (approximately 1, 5, 10, 25 and 50 µg/mL) were prepared from the secondary working standard mixes.

Spiking surrogate standard--An approximately 10 µg/mL of hexane was prepared in methanol from the stock standard. This solution was spiked in all blanks, spikes, and samples prior to analysis.

Storage and handling precautions --All solutions (except stock standards) were stored at 4°C and labeled with study number, names, concentrations, and expiration date. All solutions will be disposed of upon release of the final report

PROCEDURE:

Set up the acquisition sequence on the Waters chromatography data system.

A 5 mL Luer Lock syringe is filled to overflowing with deionized water which has also been heated to boiling to remove residual volatile organics. The plunger is replaced and the water compressed to the 5 mL mark. The plunger is pulled back slightly to allow for the addition of 5 µL of calibration standard or spiking surrogate standard. After the solution is loaded to the P&T, press START on the LSC 2000 front panel to start the purge-and-trap procedure.

Initial calibration - Run five levels of calibration standards following the procedure described above and calculate the response factor (RF) of the individual analytes based on equation (I):

$$RF = A_S/C_S \qquad (I)$$

where:

A_S: peak area count of analyte

C_S: amount in nanograms (e.g., 5 µL of a 1.0 µg/mL solution = 5 ng) of the calibration standard injected into the syringe

Calculate the average response factor (RF_{ave}) and standard deviation (SD) of five-level calibration standards. Calculate the relative standard deviation (%RSD = (SD/RF_{ave}) x 100) of the calibration using Microsoft Excel (version 4.0). If %RSD is < 20%, then the RF_{ave} of the analytes is used for quantitation. If %RSD > 20%, the first degree linear regression (forced through zero) with r> 0.99 is used for quantitation (re: quantitative analysis section).

Sample analysis - The analysis follows the steps described above. Samples were analyzed only once using one of two duplicate sample vials except when a need for further confirmation arose or when dilutions were required to bring the response of the analytes within the range of the calibration standards. The duplicate sampling vials were used in these cases.

WAF GENERATION AND EVALUATION:

Two types of WAFs of Whole Light Alkylate Product were evaluated to demonstrate equilibrium and maintenance of test material. A WAF prepared with freshwater was evaluated at 0,1,3,6,24,36,48,60 and 72 hours after preparation while a WAF prepared with saltwater was evaluated at 0,1,3,6,12,24,36 and 48 hours after preparation. The WAFs were generated following modification of the procedure used by Anderson, et al (1974, Marine Biol., 27: 75-88). Two WAFs were prepared, using each water type, containing 50 ppm of Whole Light Alkylate Product. One WAF of each water type was prepared in a bottle filled to the neck to minimize headspace ("XXX1X" sample designation, e.g, sample "3FW2B" is a 3-hour, freshwater, type 1 WAF, the second of duplicate samples collected), while the second WAF of each water type was prepared in a bottle filled to the shoulder to maximize product-water contact ("XXX2X" sample designation). Duplicate samples were collected from each bottle (except for time zero "XXX2X" series) at the specified time periods, with one sample analyzed using the methodology determined from the in-house validation and the other sample acting as a backup. All samples were collected in 40 ml glass vials with no headspace. The concentration in each flask was quantified to evaluate the consistency of the WAF with time, water type and stirring procedure.

GOOD LABORATORY PRACTICES:

This study was conducted according to the EPA Good Laboratory Practice Standards outlined in 40 CFR Part 160, Federal Register Vol. 54, No.158, 8/17/89.

Test Substance(s) Characterization - The methods of synthesis, fabrication, and/or derivation of the test materials is the responsibility of the sponsor. In addition, the stability, identity, strength, purity and composition of other characteristics which identify the test materials are the responsibility of the sponsor. The test article data are located at the sponsor's facility.

Chemical purity and stability data for reference and control standards purchased commercially, with the exception of 2,3,3-trimethylpentane and 1-methyl-ethylcyclopentane, were provided by the suppliers (Supelco, Sigma). The latter two compounds were assayed for purity at Stonybrook Laboratories Inc. These data and those provided by the suppliers are archived with the raw data.

RECORDS MAINTAINED:

The study file contains but is not limited to the following records or verified copies of:

Notice of Intent to Initiate Study
Request for Testing
Sponsor Protocol Amendment Approval Memo
Study Protocol and Amendments
Technical Personnel Records
Reagents and Equipment Inventory
Chemical Repository Unit (CRU) Dispensing Records
Study Notebook Records

RESULTS & DISCUSSION

METHOD EVALUATION/VALIDATION:

The use of the PT/GC technique for the analysis of whole light alkylate product WAF was based on a review of the test article composition and the anticipated composition of the WAF. The use of PT/GC runs throughout the EPA analytical methods series for drinking water (500), municipal/industrial effluent water (600) and wastewater (8000). The method has been tentatively validated for the analysis of gasoline range organics (GRO) in the last year and drafts of the method were made available by the Office of Solid Waste (OSW) prior to the expected promulgation in late 1994.

Six alkanes and one cycloalkane were selected (representing 68% of the components of the test material) for the in-house evaluation/validation. Hexane was chosen as the surrogate. The EPA procedure for the evaluation of method performance is an appropriate standard by which to assess in-house method validation. Determination of the method detection limit (MDL), limit of detection (LOD) and limit of quantitation (LOQ) provide an excellent measure of the sensitivity and precision of the procedure. MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The LOD is the lowest concentration that can be determined statistically differently from the blank. LOD is numerically defined as three times the standard deviation from replicate measurements of standard. LOQ is the level above which quantitative results may be obtained and is numerically defined as ten times the standard deviation from replicate measurements of standards. The LOD, LOQ and MDL were determined from replicate measurements of the analytes and surrogate in water at 5 PPB. In general, the per component MDL was slightly below 5 PPB. The LOD, LOQ and MDL for each of the compounds is reported in table I.

WAF GENERATION AND EVALUATION:

Two types of WAF were generated to evaluate the affect of mixing and headspace on final WAF concentration. The concentration of test article components was significantly higher (factor of 2) in the "minimal headspace" type WAF as compared to the "maximum phase interface" type WAF. Table II reports the time course of WAF concentration for the individual and summed seven analytes monitored for both freshwater (through 72 hours) and saltwater (through 48 hours). The surrogate recoveries, which were essentially quantitatve, are also reported for each WAF sample analyzed.

WAF concentration of test material peaked at approximately 12 hours in saltwater (0.9 PPM) and 24 hours in freshwater (1.6 PPM) using the "minimal headspace"WAF generation procedure. This can be seen more clearly in Figure 1 where the "Total" column data in table II for freshwater and saltwater WAF concentrations are plotted vs time of sampling in a histogram format. Figure 2 plots the individual component concentrations for freshwater and saltwater WAF vs sampling time and shows that the relative concentration of the individual test article WAF components is largely maintained over the mixing period. Figures 3 and 4 compare the 24 hour WAF concentration of the test article components with the actual concentration of the components in the test article. These experimentally observed results can be predicted with a reasonable degree of accuracy if the water solubility or octanol/water partition coefficients of the components are taken into consideration.

Table 1

Summary Sheet for LOD, LOQ and MDL Determinations for Whole Light Alkylate Product WAF Components and Surrogate

Peaks	Compound	Rt. (min.)	Area count						
			Run1	Run2	Run3	Run4	Run5	Run6	Run7
1	2,3-dimethylbutane	8.065	37422	31896	36712	34842	30650	20817	46749
2	hexane (surr)	8.850	35696	30159	34788	34045	28001	19625	46398
3	2,4-dimethylpentane	9.600	41098	34678	40270	37909	33107	22357	52058
4	2,2,4-trimethylpentane	11.420	43558	36274	41736	41538	36182	25682	54862
5	2,3-dimethylhexane	12.750	40754	34303	39308	40101	34222	24153	51361
6	2,3,4-trimethylpentane	13.480	41512	35834	41050	42265	37296	25760	53336
7	2,3,3-dimethylpentane	13.680	41454	36507	41116	42461	38096	35948	41716
8	1-methyl-1-ethylcyclopentane	15.115	46856	42044	46961	49500	44715	45595	47700

Peaks	Compound	Rt. (min.)	PFSCL ODLOQ R1	PFSCL ODLO QR2	PFSCL ODLO QR3	PFSCL ODLOQ R4	PFSCL ODLOQ R5	PFSCL ODLO QR6	PFSCL ODLO QR7	RF(ave)	Std. Dev.	%RSD	Std. Dev. (ppb)	LOD (ppb)	LOQ (ppb)	t value*	MDL (ppb) =Std. Dev. x t
			Response factor=Area count/5 (ng)														
1	2,3-dimethylbutane	8.065	7484.4	6379.2	7342.4	6968.4	6130.0	4163.4	9349.8	6831.1	1573.9	23.0	1.2	3.5	12	3.71	4.3
2	hexane (surr)	8.850	7139.2	6031.8	6957.6	6809.0	5600.2	3925.0	9279.6	6534.6	1637.4	25.1	1.3	3.8	13	3.71	4.6
3	2,4-dimethylpentane	9.600	8219.6	6933.6	8054.0	7581.8	6621.4	4471.4	10411.6	7470.8	1905.8	24.2	1.2	3.6	12	3.71	4.5
4	2,2,4-trimethylpentane	11.420	8711.6	7254.8	8347.2	8307.6	7236.4	5136.4	10972.4	7995.2	1774.6	22.2	1.1	3.3	11	3.71	4.1
5	2,3-dimethylhexane	12.750	8150.8	6860.8	7861.6	8020.2	6844.4	4830.6	10272.2	7548.6	1656.2	21.9	1.1	3.3	11	3.71	4.1
6	2,3,4-trimethylpentane	13.480	8302.4	7166.8	8210.0	8453.0	7459.2	5152.0	10667.2	7915.8	1658.5	21.0	1.0	3.1	10	3.71	3.9
7	2,3,3-dimethylpentane	13.680	8290.8	7301.4	8223.2	8492.2	7619.2	7189.6	8343.2	7922.8	538.9	6.80	0.34	1.0	3.4	3.71	1.3
8	1-methyl-1-ethylcyclopentane	15.115	9371.2	8408.8	9212.2	9500.0	8943.0	9119.0	9540.0	9213.5	471.2	5.11	0.26	0.77	2.6	3.71	0.95

* t value at 99% confidence interval

Table II
Continued

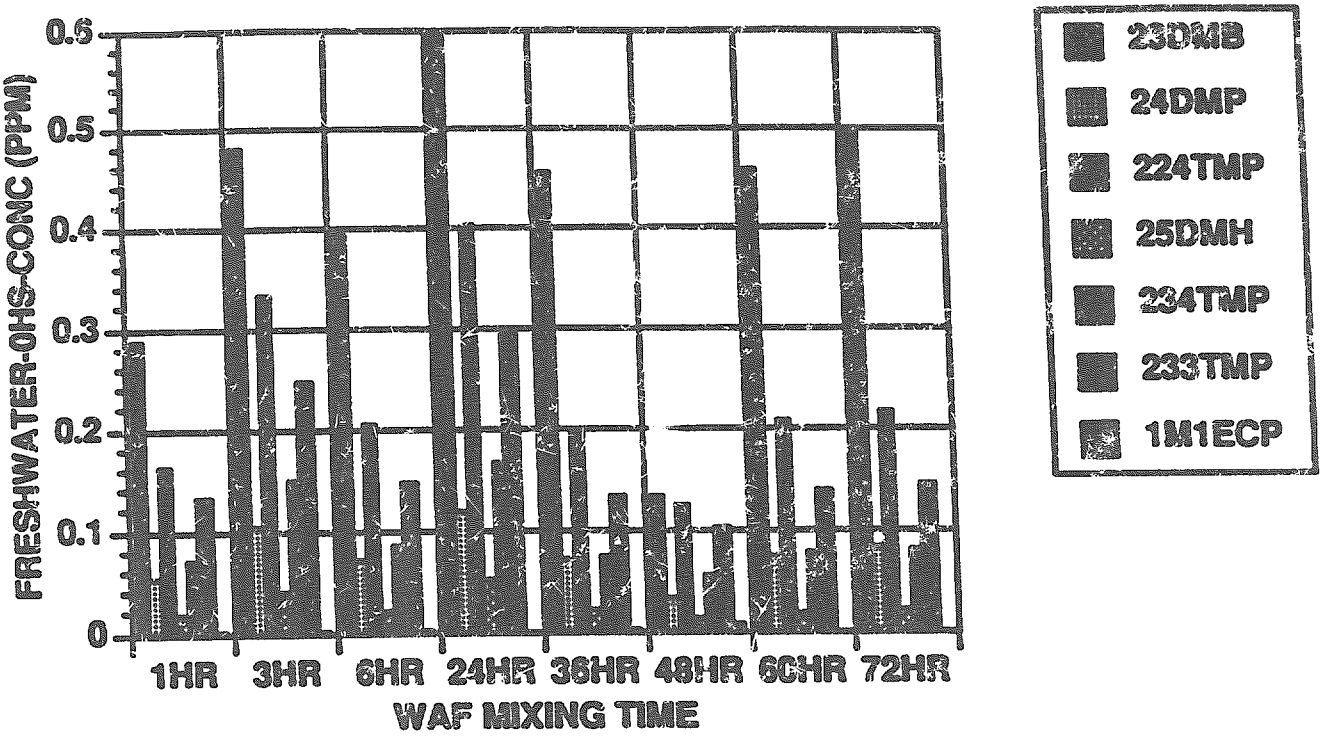
		2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total	hexane (surr) recovery (%)
	RF(ave)	*7524.2*	*8025.4*	*8559.9*	*8495.5*	*8756.4*	*8818.6*	*9204.0*		*7378.6*
Data file	DF*									
36FW1A	20	0.455	0.072	0.197	0.022	0.073	0.133	0.002	0.953	111
36SW1A	10	0.372	0.056	0.145	0.016	0.055	0.101	0.002	0.747	114
36FW2A	20	0.206	0.041	0.128	0.014	0.055	0.103	0.000	0.547	109
36SW2A	10	0.174	0.036	0.117	0.014	0.052	0.100	0.000	0.492	102
48FW1A	20	0.132	0.031	0.125	0.016	0.054	0.102	0.006	0.465	110
48SW1A	10	0.327	0.049	0.134	0.013	0.050	0.099	0.000	0.672	109
48FW2A	20	0.331	0.062	0.186	0.019	0.074	0.140	0.000	0.812	81
48SW2A	10	0.166	0.033	0.100	0.012	0.044	0.084	0.000	0.438	114
60FW1A	20	0.456	0.074	0.206	0.021	0.076	0.138	0.000	0.972	102
60FW2A	20	0.242	0.055	0.184	0.024	0.082	0.146	0.000	0.733	109
72FW1A	20	0.500	0.081	0.216	0.023	0.079	0.143	0.000	1.042	109
72FW2A	20	0.222	0.046	0.142	0.016	0.059	0.110	0.000	0.595	101

*dilution factor

Data file format - e.g., 36FW1A = 36 hour collection time, freshwater, type "1" WAF (see experimental section), "A", first of two (duplicate) samples collected at the indicated time point.

Figure 2

Individual Monitored Component Concentrations in Whole Light Alkylate Product Freshwater and Saltwater WAFs over 48-72 Hours



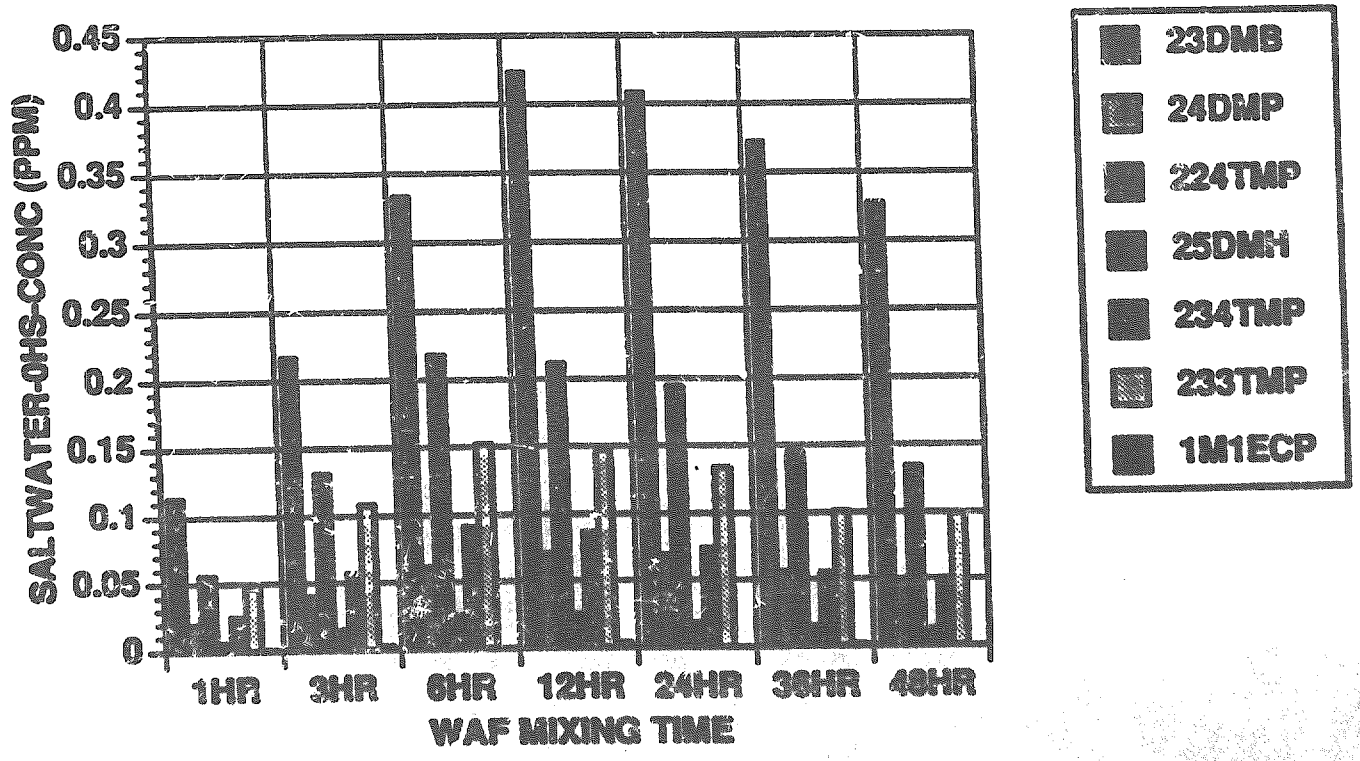
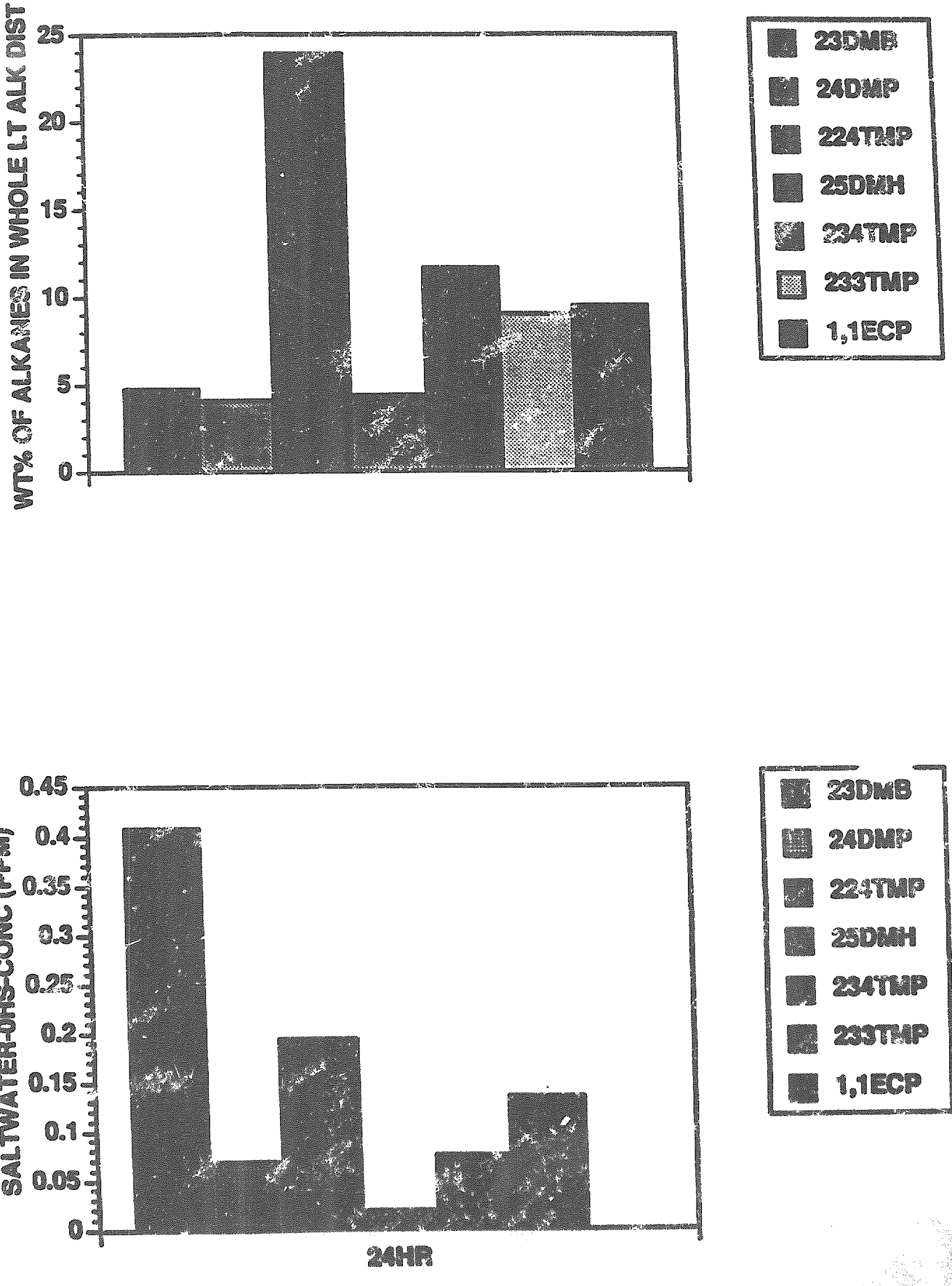


Figure 4

Study No. 65969

Comparison of Whole Light Alkylate Product Alkane Concentrations In The Neat Material With Their 24 Hour WAF Concentration (Saltwater)

65969 10/26/94 DATA

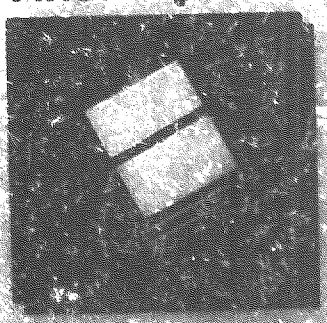


Stonybrook
Laboratories Inc.

Static-Renewal 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to Fathead Minnow

Stonybrook Laboratories Inc.
Princeton, NJ

Final Report



Study Number 65908

STONYBROOK LABORATORIES INC.
REPORT RELEASE

TO STUDY DIRECTOR/LIAISON: C.A. Schreiner
STUDY NUMBER: 65908
CRU NUMBER: 94194
SAMPLE NAME: Whole Light Alkylate Product
STUDY TITLE: Static-Renewal 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to Fathead Minnow
REQUESTER: Petroleum Product Stewardship Council

RESULTS:

LC_{50} 8.2 ppm for Whole Light Alkylate Product (nominal)
LC_{50} 305 ppb for Whole Light Alkylate Product (measured)

A static-renewal 96-hour toxicity study was conducted December 16-20, 1994 to determine the acute toxicity of Whole Light Alkylate Product to fathead minnow, a representative freshwater fish species. Test fish were exposed to individual water accommodated fractions (WAFs) of the poorly water-soluble test material at nominal concentrations of 1.1 ppm, 5.2 ppm, 9.7 ppm, 19 ppm, and 74 ppm (w/v, based on density). Nominal test concentrations are based on the loading rate, or amount of test material added to make each WAF. Test solutions were renewed at 24 hour intervals during conduct of the study. Water quality parameters of pH, temperature, and dissolved oxygen (D.O.) were measured during the study.

Samples of the control and exposure concentrations were collected daily and quantitatively analyzed using gas chromatography (GC). The concentrations were quantitated by GC using seven Whole Light Alkylate Product component standards. Measured test concentrations are based on the total concentration of all analytes. Test material retention from the static-renewal procedure ranged from 71.7->100.0%, producing consistent exposure of the test fish to Whole Light Alkylate Product throughout the study.

The toxicity of the test material was evaluated on the basis of LC_{50} determinations at 24, 48, 72, and 96 hours. The term LC_{50} used in this report refers to the concentration causing 50% mortality after a specified exposure period. The computer-estimated 96-hour LC_{50} for Whole Light Alkylate Product to fathead minnow under static-renewal test conditions was 8.2 ppm based on nominal exposure concentrations, and 305 ppb based on measured exposure concentrations. The 96-hour no observed effect concentration (NOEC), based on nominal concentrations, was 5.2 ppm, since exposure to concentrations of 9.7 ppm and greater resulted in significant mortality. The 96-hour no observed effect concentration (NOEC), based on measured concentrations, was 164 ppb, since exposure to concentrations of 364 ppb and greater resulted in significant mortality.

Approvals:

J.F. Barbieri 12/1/95
Study Director/Date
J.F. Barbieri

M.T. BenKinney 12/1/95
Supervisor/Date
M.T. BenKinney

C.R. Mackerer 12/1/95
President/Date
C.R. Mackerer

Distribution: Study Director, Liaison, Archives (Original)

RESULTS & DISCUSSION

METHOD EVALUATION/VALIDATION:

The use of the PT/GC technique for the analysis of whole light alkylate product WAF was based on a review of the test article composition and the anticipated composition of the WAF. The use of PT/GC runs throughout the EPA analytical methods series for drinking water (500), municipal/industrial effluent water (600) and wastewater (8000). The method has been tentatively validated for the analysis of gasoline range organics (GRO) in the last year and drafts of the method were made available by the Office of Solid Waste (OSW) prior to the expected promulgation in late 1994.

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Table 1

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8	1-methyl-1-ethylcyclopentane	15.115	46856	42044	46061	49500	44715	45593	47700

Peak#	Compound	Rt. (min.)	PFSCL ODLOQ R1	PFSCL ODLO QR2	PFSCL ODLO QR3	PFSCL ODLOQ R4	PFSCL ODLOQ R5	PFSCL ODLO QR6	PFSCL ODLO QR7
			Response factor=Area count/5 (ng)						
1	2,3-dimethylbutane	8.465	7484.4	6379.2	7342.4	6968.4	6130.0	4163.4	9349.8
2	hexane (surr)	8.850	7133.2	6031.8	6957.6	6809.0	5600.2	3925.0	9279.6
3	2,4-dimethylpentane	9.600	8219.6	6935.6	8054.0	7581.8	6621.4	4471.4	10411.6
4	2,2,4-trimethylpentane	11.420	8711.6	7254.8	8347.2	8307.6	7236.4	5136.4	10972.4
5	2,3-dimethylhexane	12.750	8150.8	6860.6	7861.6	8020.2	6844.4	4830.6	10272.2
6	2,3,4-trimethylpentane	13.480	8302.4	7164.8	8216.0	8453.0	7459.2	5152.0	10667.2
7	2,3,3-trimethylpentane	13.680	8290.8	7301.4	8223.2	8492.2	7619.2	7189.6	8343.2
8	1-methyl-1-ethylcyclopentane	15.115	9371.2	8408.8	9212.2	9900.0	8943.0	9119.0	9540.0

RF(ave)	Std. Dev.	%RSD	Std. Dev. (ppb)	LOD (ppb)	LOQ (ppb)	t value*	MDL (ppb) =Std. Dev. x t
6831.1	1573.9	23.0	1.2	3.5	12	3.71	4.3
6534.6	1637.6	25.1	1.3	3.8	13	3.71	4.6
7470.8	1805.8	24.2	1.2	3.6	12	3.71	4.3
7995.2	1774.6	22.2	1.1	3.3	11	3.71	4.1
7548.6	1656.2	21.9	1.1	3.3	11	3.71	4.1
7915.8	1658.5	21.0	1.0	3.1	10	3.71	3.9
7922.8	538.9	6.80	0.34	1.0	3.4	3.71	1.3
9213.5	471.2	5.11	0.26	0.77	2.6	3.71	0.95

* t value at 99% confidence interval

Table II
Continued

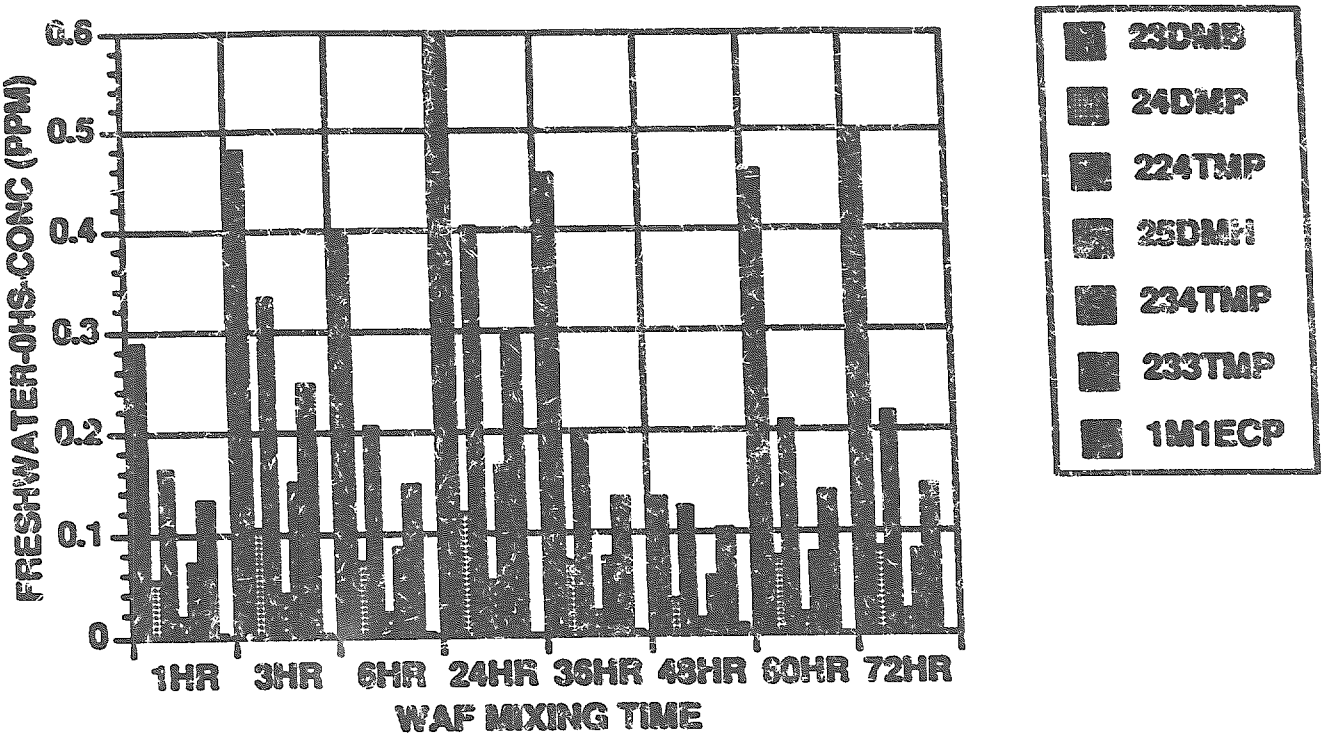
		2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total	hexane (surr) recovery (%)
	RF(ave)	*7524.2*	*8025.4*	*8569.9*	*8495.5*	*8756.4*	*8818.6*	*9204.0*		*7378.6*
Data file	DF*									
36FW1A	20	0.455	0.072	0.197	0.022	0.073	0.133	0.002	0.953	111
36FW1A	10	0.372	0.056	0.145	0.016	0.055	0.101	0.002	0.747	114
36FW2A	20	0.206	0.041	0.128	0.014	0.055	0.103	0.000	0.547	109
36FW2A	10	0.174	0.036	0.117	0.014	0.052	0.100	0.000	0.492	102
48FW1A	20	0.132	0.031	0.125	0.016	0.054	0.102	0.006	0.465	110
48FW1A	10	0.327	0.049	0.134	0.013	0.050	0.099	0.000	0.672	109
48FW2A	20	0.331	0.062	0.186	0.019	0.074	0.140	0.000	0.812	81
48FW2A	10	0.166	0.033	0.100	0.012	0.044	0.084	0.000	0.438	114
60FW1A	20	0.456	0.074	0.206	0.021	0.076	0.138	0.000	0.972	102
60FW2A	20	0.242	0.055	0.184	0.024	0.082	0.146	0.000	0.733	109
72FW1A	20	0.500	0.081	0.216	0.023	0.079	0.143	0.000	1.042	109
72FW2A	20	0.222	0.046	0.142	0.016	0.059	0.110	0.000	0.595	101

*dilution factor

Data file format - e.g., 36FW1A = 36 hour collection time, freshwater, type "1" WAF (see experimental section), "A", first of two (duplicate) samples collected at the indicated time point.

Figure 2

Individual Monitored Component Concentrations in Whole Light Alkylate Product Freshwater and Saltwater WAFs over 48-72 Hours



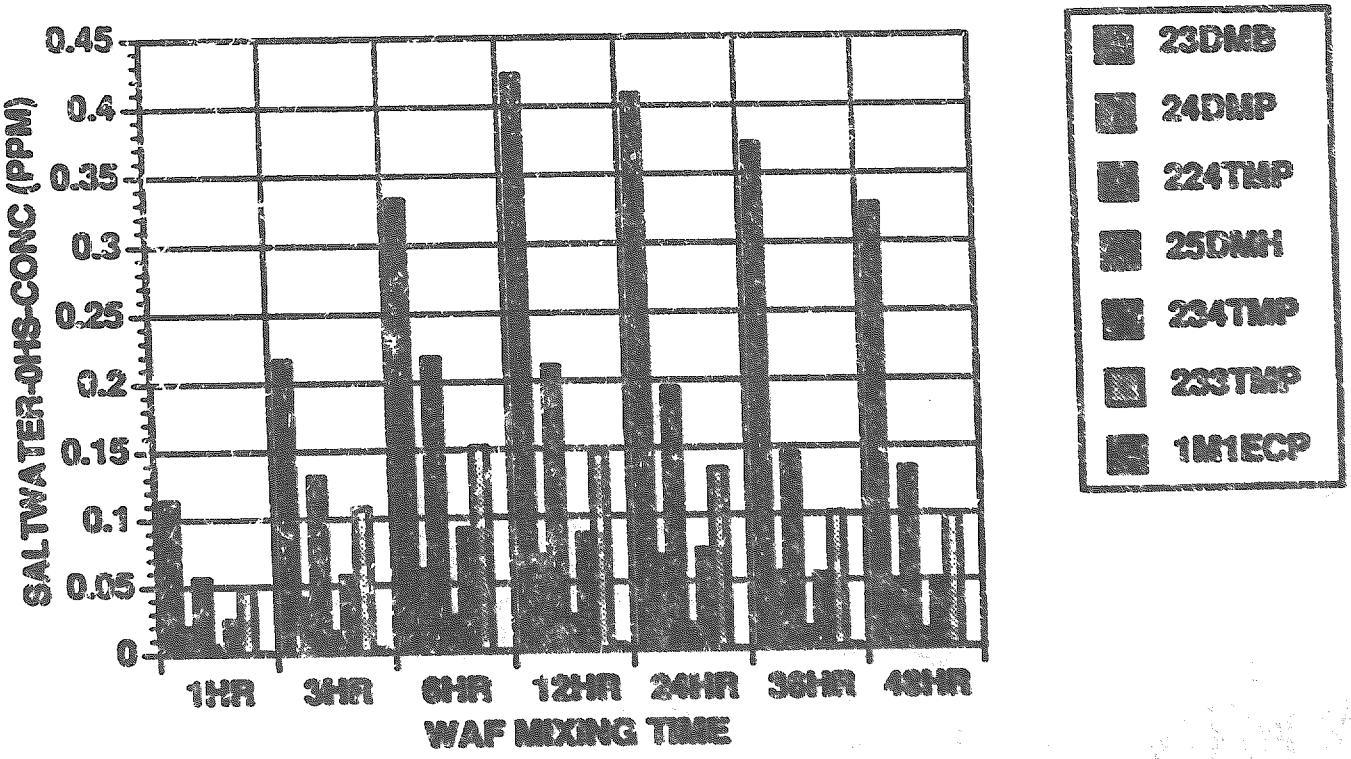
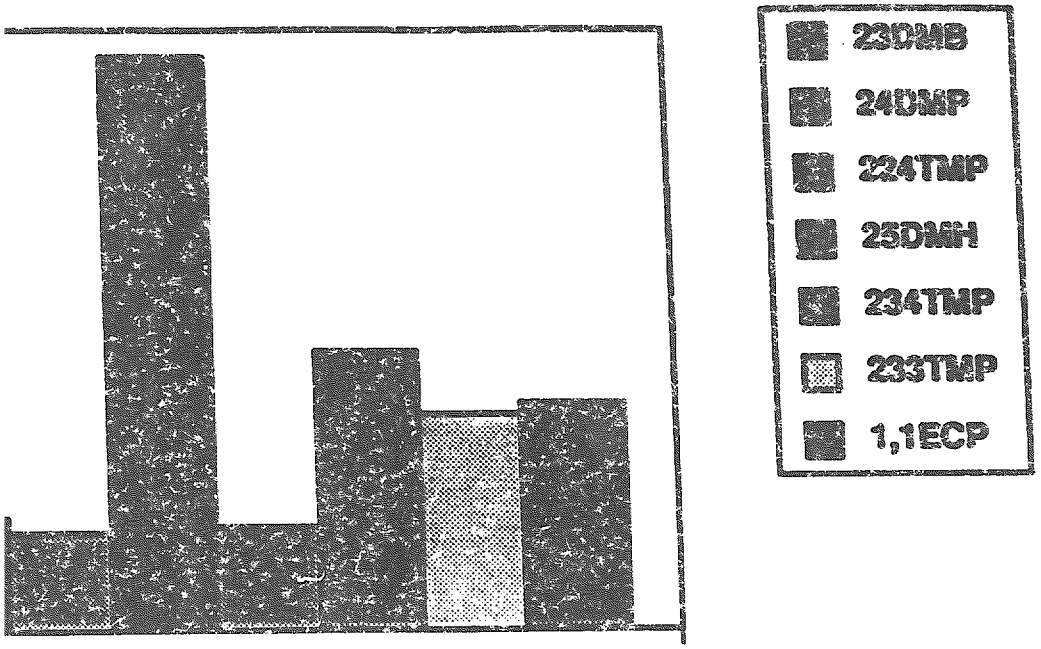
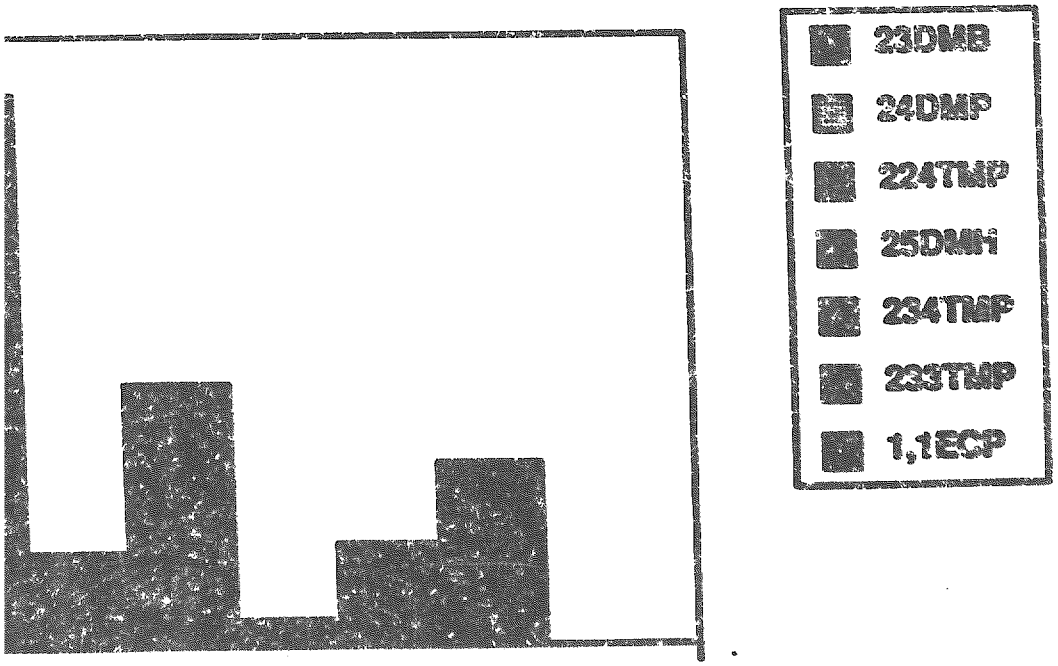


Figure 4

of Whole Light Alkylate Product Alkane Concentrations In
terial With Their 24 Hour WAF Concentration (Saltwater)

969 10/26/94 DATA





24HR

STATIC-RENEWAL 96-HOUR ACUTE TOXICITY STUDY OF THE WATER ACCOMMODATED FRACTION (WAF) OF WHOLE LIGHT ALKYLATE PRODUCT TO FATHEAD MINNOW

STUDY No.: 65908

MATERIAL TESTED:	Whole Light Alkylate Product
CRU SAMPLE No.:	94194
REQUESTER:	Petroleum Product Stewardship Council c/o Synthetic Organic Chemical Manufacturing Association 1100 NY Ave., NW, Suite 1090 Washington, D.C. 20005
STUDY PERFORMED BY:	Stonybrook Laboratories Inc. 311 Pennington-Rocky Hill Road Pennington, N.J. 08534
STUDY INITIATION DATE:	July 22, 1994
EXPERIMENTAL START DATE:	November 8, 1994
EXPERIMENTAL TERMINATION DATE:	January 4, 1995

Compliance Statement

Study No. 65908

This study was conducted according to the USEPA Toxic Substances Control; Good Laboratory Practice Standards. 40 CFR Part 792, except as noted below; the final report fully and accurately reflects the raw data generated in the study.

Exceptions to GLPs:

1. The test material, Whole Light Alkylate Product, was not characterized and stability analysis was not performed at this facility.

2. Some data entries were made late. These late entries were indicated as such.

3. Some equipment logs were not up to date at the time of the study.

J. F. Barbin/MBK 12/1/95

Study Director Date

STONYBROOK LABORATORIES INC.

QUALITY ASSURANCE STATEMENT

Study Number: 65908

Title of Study: Static-Renewal 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to Fathead Minnow

Listed below are the dates that this study was reviewed by the Quality Assurance Unit and the dates that the findings were reviewed by the Study Director and Management.

DATE(S) OF QA REVIEW	PHASE OF STUDY	DATE(S) REVIEWED BY STUDY DIRECTOR	DATE(S) REVIEWED BY MANAGEMENT
11/15/94	PROTOCOL REVIEW	11/28/94	1/23/95
11/17/94	IN-PROCESS INSPECTION	1/6/95	1/9/95
12/19/94	IN-PROCESS INSPECTION	2/19/95	2/25/95
3/28-31/95	DATA REVIEW	4/19/95	4/21/95
4/5/95	FINAL REPORT AUDIT	4/19/95	7/18/95

Lisa Meck
Manager, Quality Assurance

7/20/95
Date

PRINCIPAL INVESTIGATOR

STUDY DIRECTOR 12/1/95

* no longer with Company MTB 12/1/95

DISTRIBUTION:

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Study Director:	J.F. Barbieri, B.S.
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Additional Personnel Involved In The Study

N.A. Afonina : Laboratory Technician

C.W. Chuang : Study Chemist

A.L. Crawford : Laboratory Assistant

A.L. McClurg : Laboratory Technician

A.L. Wagstaff : Laboratory Technician

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SUMMARY:

A static-renewal 96-hour toxicity study was conducted December 16-20, 1994 to determine the acute toxicity of Whole Light Alkylate Product to fathead minnow, a representative freshwater fish species. Test fish were exposed to individual water accommodated fractions (WAFs) of the poorly water-soluble test material at nominal concentrations of 1.1 ppm, 5.2 ppm, 9.7 ppm, 19 ppm, and 74 ppm (w/v, based on density). Nominal test concentrations are based on the loading rate, or amount of test material added to make each WAF. Test solutions were renewed at 24 hour intervals during conduct of the study. Water quality parameters of pH, temperature, and dissolved oxygen (D.O.) were measured during the study.

Samples of the control and exposure concentrations were collected daily and quantitatively analyzed using purge-and-trap/gas chromatography (GC). The concentrations were quantitated by GC using seven Whole Light Alkylate Product component standards. Measured test concentrations are based on the total concentration of all analytes. Test material retention from the static-renewal procedure ranged from 72.3->100%, producing consistent exposure of the test fish to Whole Light Alkylate Product throughout the study.

The toxicity of the test material was evaluated on the basis of LC_{50} determinations at 24, 48, 72, and 96 hours. The term LC_{50} used in this report refers to the concentration causing 50% mortality after a specified exposure period. The computer-estimated 96-hour LC_{50} for Whole Light Alkylate Product to fathead minnow under static-renewal test conditions was 8.2 ppm based on nominal exposure concentrations, and 305 ppb based on measured exposure concentrations. The 96-hour no observed effect concentration (NOEC), based on nominal concentrations, was 5.2 ppm, since exposure to concentrations of 9.7 ppm and greater resulted in significant mortality. The 96-hour no observed effect concentration (NOEC), based on measured concentrations, was 164 ppb, since exposure to concentrations of 384 ppb and greater resulted in significant mortality.

INTRODUCTION:

The objective of this study was to determine the acute toxicity of Whole Light Alkylate Product to aquatic organisms by evaluating its effect on fathead minnow (*Pimephales promelas*), a representative freshwater fish species. Fathead minnow were selected since they are a freshwater test species recommended in U.S. EPA (1) regulations. Static-renewal testing of the water accommodated fraction (WAF) in closed containers with as little headspace as possible was chosen as the most appropriate study design, due to the volatile nature of the test material. Under WAF exposure conditions, toxic effects from the soluble components of the test material are evaluated.

The analytical standards chosen to evaluate the WAF of Whole Light Alkylate Product were selected as representative of the alkane and cycloalkane constituents which account for 68% of the test material. These constituents were expected to be found in the highest concentrations in the WAF and account for most, if not all, of the toxicity measured during the study.

In acute toxicity tests, the most commonly used adverse effect criterion is death of the organism. Mortality data collected during the study are used to calculate an LC_{50} (concentration lethal to 50% of the test population after a specific time period which is typically 96 hours).

METHODS AND MATERIALS:

Test Fish:

Juvenile fathead minnow (*Pimephales promelas*) used in the study documented in this report were hatched and raised in-house from breeder populations. The fish were held in-house in a glass tank filled with Mobil Technical Center (MTC) well water (Table 1). Acclimation prior to experimentation lasted a minimum of 14 days on a 16-hour light/8-hour dark cycle (fluorescent lighting) following acceptable culturing techniques (2,3,4,5). The fish were fed a commercial fish food (Wardley's basic flake) and Artemia *ad libitum* during acclimation. Temperature in the holding tank was maintained from 17.5-27.5 °C during the growth and acclimation period and mortality of the test population was <5% in the 48 hours prior to study initiation. The fathead minnow were not fed in the 24 hours preceding study initiation nor during conduct of the study. Since individual identification of the fish was not possible, fathead minnow were netted and arbitrarily added to each test chamber. All fish used in the study were weighed and measured after test termination (Table 2).

Test System:

The Whole Light Alkylate Product static-renewal toxicity study was conducted in labeled 3.8 liter glass jars, sealed with teflon lined screw caps. The labeling of the test jars included the study number, CRU number, test date, concentration, group number, replicate letter, and species designation. Test jars contained 3.8 liters of test solution, allowing no headspace. The water source for the study was MTC well water. The test exposure chambers were held in a water bath maintained at 22 ± 1 °C. The photoperiod during testing was 16-hr light/8-hr dark (fluorescent lighting).

The fathead minnow were exposed to individual WAF solutions of Whole Light Alkylate Product. Generation of the WAF solutions was produced following a modification of the procedure used by Anderson, et al., 1974 (6). Approximately twenty-five hours prior to test initiation, six individual WAF 9 liter bottles were set up. A stir bar and 9.4 liters of test water were placed into each bottle. A 9 liter bottle filled to the neck (instead of the normal shoulder height) can hold 9.4 liters. The bottles were filled to neck height to minimize volatility. A measured amount of Whole Light Alkylate Product (nominal concentration), calculated for each exposure concentration, was added into each bottle. All bottles were capped tightly with a positive pressure siphoning apparatus and parafilm. The siphoning apparatus was comprised of a teflon lined neoprene stopper housing two teflon tubes. One siphon tube extended to the bottom of the WAF solution The other tube ended above the WAF surface, and was used to control air pressure while siphoning. The stirring speed of the bottles was adjusted to produce a vortex of less than 25% of the container depth. The solutions stirred for approximately 24 hours, and then were allowed to settle for approximately 5 minutes to one hour except at study initiation when the solutions settled for approximately 45 minutes to less than 2 hours. After the stirring/settling period, the aqueous phase (WAF) was siphoned using positive air pressure. Two 3.8 liter replicates were prepared from each individual WAF. A sample was also collected to take initial water quality measurements. Duplicate 40 ml samples were also taken of the WAF for chemical analysis. The solution in each test container was renewed daily during the study. The renewal concentrations were produced in the same manner as the initial concentrations. The test fish remained in the test container during the renewal process.

Test Material:

The test material, Whole Light Alkylate Product, was dispensed by Stonybrook Laboratory's Chemical Repository Unit (CRU) from a homogeneous sample obtained from the sponsor. As reported in the Product Physical and Chemical Data (PPCD) sheet, Whole Light Alkylate Product (CRU No. 94194) consists entirely of Light Alkylate Naphtha. It was received as a liquid. The stability, identity, strength, purity, and composition or other

characteristics which identified the test material was the responsibility of the sponsor. The concentrations used in this study were prepared by pipetting known quantities into each WAF bottle on a weight to volume basis, based on the density (0.7 g/ml) of the test material. Following a stirring and settling period, the aqueous phase of each solution was used for its corresponding exposure concentration.

Test Procedure-Biological:

A range finding test was performed November 8-10, 1994, to assess the toxicity of the test material under closed container static renewal conditions. This range finding study consisted of 10 fish per replicate exposed to a control and three concentrations of 0.97 ppm, 9.7 ppm, and 97 ppm evaluated in duplicate. At test termination, no mortality was observed in the control or 0.97 ppm concentration, with insignificant mortality (1 fish, 5%) in the 9.7 ppm concentration. Also at 48 hours, total mortality was observed in the 97 ppm concentration. Based on these results, a dose range of 9-149 ppm was chosen for the definitive study.

An initial 96-hour definitive study was conducted November 14-18, 1994, consisting of a control and test concentrations of 9 ppm, 19 ppm, 37 ppm, 74 ppm, and 149 ppm, evaluated in duplicate. This study was conducted using a static-renewal test procedure, with daily replacement of solution in each test chamber. At test termination, insignificant mortality (1 fish, 5%) was observed in the control. Also at 96 hours, total mortality was found in the 19 ppm, 74 ppm and 149 ppm concentrations, with partial mortality in the 9 ppm (17 fish, 85%) and 37 ppm (19 fish, 95%) concentrations. Since all exposure concentrations produced greater than 50% mortality, the 96-hour LC_{50} was <9 ppm. A second definitive run was conducted, with a lower dose range, to produce an actual LC_{50} value.

The 96-hour definitive toxicity study documented in this report was conducted December 16-20, 1994. This study was conducted using a static-renewal test procedure, with daily replacement of solution in each test chamber. All concentrations were run in duplicate in 3.8 liter glass jars containing 3.8 liters of solution, with no headspace. Fathead minnow were arbitrarily added, two at a time, until each replicate contained 10 fish, within one hour of initial WAF solution preparation. The test chambers were held in a water bath (20 ± 2 °C), and sealed with teflon lined screw caps to minimize volatilization. Exposure concentrations with surviving fish were renewed at each 24-hour interval during conduct of the study by siphoning the final solutions out of each test chamber, leaving only enough volume so that the organisms were not distressed. Approximately one hundred (100) ml of each final solution was retained for water quality analysis. A composite 40 ml final sample of each concentration (20 ml for each replicate) was also taken at the renewal for chemical analysis except the final 96 hour sample for the highest concentration where only one replicate was sampled (the other replicate showed total mortality at the previous 24 hour observation period. The newly prepared solution was then carefully siphoned into each test chamber.

The fish were exposed to a control and five nominal concentrations (1.1 ppm, 5.2 ppm, 9.7 ppm, 19 ppm, and 74 ppm) of Whole Light Alkylate Product. The control consisted of the same dilution water, test conditions, and test organisms with no added test material. The fish in each test chamber were observed daily for mortality at 1, 3, 6, and 24 hour intervals. Daily observations at 1, 3, and 6 hours were made with the jar lids remaining on, to prevent volatilization. The 24, 48, 72 and 96 hour observations were made with the lids removed, during renewal or at termination. Abnormalities such as surfacing, coughing, loss of equilibrium and discoloration were documented, if observed, at each observation period. The criterion for death was a lack of opercular movement. Fish remaining alive at the end of the study were killed by an anesthetic overdose of approximately 200 ppm Finquel® solution, placed in labeled plastic bags, and frozen prior to measurement.

Test Procedure-Water Quality Analysis:

Water quality parameters of dissolved oxygen (D.O.), pH, and temperature were measured at study initiation and daily in a portion of the freshly-prepared initial samples. These water quality parameters were also taken daily in final replicate samples. Water quality was performed only on final samples from test chambers that contained some living organisms at the previous 24 hour observation period, and in initial samples from chambers with some living organisms present. Dissolved oxygen was measured with a YSI Model 57 D.O. Meter with a Model 5739 D.O. probe. The pH was measured with an Orion Model 520A Digital pH/mV Meter with an Orion Model 81-02 Combination pH Electrode. Temperature was measured with a hand-held thermometer, with a stainless steel thermocouple.

Test Procedure-Chemical Analysis:

Chemical analysis was performed on single 40 ml samples, both initial and final, of the control and all exposure concentrations at 0, 24, 48, 72, and 96 hours after test initiation. Chemical analysis was performed only on final samples from test chambers that contained some living organisms at the previous 24 hour observation period, and in initial samples from chambers with some living organisms present. The samples were collected in 40 ml jars with no head space, and transferred to the Analytical Chemistry group for analysis. The concentration of Whole Light Alkylate Product in each sample was determined by using purge-and-trap and a gas chromatograph equipped with a flame ionization detector (GC-FID) following the guidelines of the methods validation study (Study 65969). Details of the method are included in the appendix. The following components of Whole Light Alkylate Product were quantified: 2,3-dimethyl butane, 2,4-dimethyl pentane, 2,2,4-trimethyl pentane, 2,5-dimethyl hexane, 2,3,4-trimethyl pentane, 2,3,3-trimethyl pentane, and 1-methyl-1-ethyl-cyclopentane. Based on the method validation study, these components represent 68% of the composition of Whole Light Alkylate Product. All chemical analysis (summary in Analytical Chemistry Report, raw data in Appendix 3) was performed by C.W. Chuang of the Analytical Chemistry Group.

Statistical Analysis:

Daily LC_{50} values were calculated on the basis of mortality data and nominal/measured dose levels. Statistical analysis of the data was calculated by a computer software LC_{50} program developed by Stephan et al. (7). This program statistically calculates the EC_{50} using binomial probability analysis, moving average angle analysis, and probit analysis. The LC_{50} was also calculated using the Spearman-Karber method (8,9). These different methods of analyzing the data are used since no one method of analysis is appropriate for all possible sets of data that may be obtained (10). The no observed effect concentration values were calculated using Fisher's exact test (9). The method selected for analysis of the data present in this report was determined by the characteristics of the data base.

Daily measured dose levels, for each concentration, were a cumulative total of all sample values evaluated between the 0 hour initial sample and the final sample, inclusive, for that time period. Measured dose levels were the cumulative total of all measured test material components, for each concentration. In cases where the measured component levels were below that component's detection limit, a zero value was included in the addition of components. The detection limits used were determined in the methods validation study. For the 96 hour time period (all samples), a standard deviation was also calculated. The average measured levels for each time period were used along with corresponding survival data to produce measured LC_{50} and NOEC values. Also for each concentration, all initial sample values were averaged, and all final sample values were averaged. The percent difference between initial and final averages was used to calculate the average percent retention at each exposure.

Data Storage:

The study was conducted according to the EPA Good Laboratory Practice Standards (40 CFR Part 792) (11). Raw data (Appendix 3) and the original final report are maintained in the Archives of Stonybrook Laboratories Inc. located in Pennington, New Jersey.

RESULTS:

The LC_{50} values for the 96-hour static-renewal toxicity study of Whole Light Alkylate Product to fathead minnow (*Pimephales promelas*) are summarized in Table 3. The 24 and 48 hour LC_{50} values were both 19.6 ppm, while the 72 and 96 hours values were both 8.2 ppm, based on nominal exposure concentrations. Based on daily measured exposure concentrations, the 24, 48, 72, and 96 hour LC_{50} values were 553 ppb, 494 ppb, 323 ppb, and 305 ppb, respectively. All LC_{50} values were determined by binomial probability analysis. Cumulative mortality data for this study are presented in Table 4. Behavioral observations are presented in Table 5.

Water quality parameters of pH, dissolved oxygen, and temperature were performed only on final samples from test chambers that contained some living organisms at the previous 24 hour observation period, and in initial samples from chambers with some living organisms present. Mean values and the range/standard deviation for each test chamber are summarized in Table 6 and 7.

The measured concentrations of Whole Light Alkylate Product in the test chambers were determined by purge-and -trap/gas chromatography (Appendix 1). The concentrations listed in this appendix are based on the coding system where the first character represents the test concentration group as listed in the protocol; the second character represents either an initial (I) or a final (F) sample; and the third and fourth characters represent the hour of the sampling period. The measured exposure concentrations and calculated averages of the samples collected during the study and the percent retention for average initial and final samples collected during the study are summarized in Tables 8 and 9. The chemical analysis techniques used in this study were developed during the Methods Validation Study (Study 65969). A copy of this study is provided in Appendix 2.

DISCUSSION:

The temperature monitored during the study remained within acceptable limits. The pH values remained consistent among concentrations and dissolved oxygen levels remained above 60% saturation in all doses. A pretest sample of 10 fish were collected at study initiation, but were not measured, however test organism loading rates were acceptable. Water quality analysis of alkalinity, hardness, and conductivity were not taken at the desired times. Water quality analysis was also not taken at 24 hours in the final sample of the highest concentration.

No mortality or behavioral abnormalities were observed in the control chambers or the two lowest test concentrations throughout the study. Total mortality was observed in the highest concentration, 74 ppm, by 24 hours. At test termination, no mortality was observed in the 1.1 ppm and 5.2 ppm concentrations. Also at test termination, partial mortality was observed in the 9.7 ppm concentration (15 fish, 75%), with total mortality in the 19 ppm concentration. Behavioral abnormalities were observed in the three highest test concentrations (9.7, 19 and 74 ppm). These abnormalities included quiescence, surfacing, twitching, hyperexcitement, erratic or ceased swimming, 90° (on side) swimming, and rapid or slow respiration. The 96-hour LC_{50} for Whole Light Alkylate Product to fathead minnow under static-renewal test conditions was, therefore, 8.2 ppm based on nominal exposure concentrations, and 305 ppb based on measured mean exposure concentrations. The 96-hour no observed effect concentration (NOEC), based on nominal concentrations, was 5.2 ppm, since exposure to concentrations of 9.7 ppm and greater resulted in significant mortality. The 96-hour no observed effect concentration (NOEC), based on measured concentrations, was 164 ppb, since exposure to concentrations of 384 ppb and greater resulted in significant mortality.

A notable trend deviation occurred at the 9.7 ppm and 19 ppm concentrations during the first 48 hours of the study. At 24 hours, there was 90% survival in the 19 ppm concentration, with 30% survival in the 9.7 ppm concentration. At 48 hours, there was 70% survival in the 19 ppm concentration, with 25% survival in the 9.7 ppm concentration. By the 72 hour observation period, 5% survival was observed in the 19 ppm concentration, with 25% survival in the 9.7 ppm concentration. Both the 72 and 96 hour observations produced an expected dose response. Chemical analysis showed that the 9.7 nominal concentration actually contained a higher measured amount of test material (817 ppb) than the 19 ppm concentration (766 ppb) at the initial 0 hour sampling period. This reversal may have contributed to the trend deviation seen in these containers.

Samples of the control and exposure concentrations were collected daily and quantitatively analyzed using purge-and-trap/gas chromatography (GC). The concentrations were quantitated using standard Whole Light Alkylate Product component standards. Test material retention from the static-renewal procedure ranged from 71.7->100%. Daily initial measured concentrations indicated consistent exposure of the test fish to Whole Light Alkylate Product throughout the study. Test material (6-16 ppb) was quantified in some of the control samples, but these amounts did not affect control survival.

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TABLE 1: Characteristics of MTC Well Water (2 Year Average)

Parameter Measured	Concentration
Dissolved Oxygen	5.2 ppm
pH	7.52
Conductivity	444 µmhos
Total Hardness ($CaCO_3$)	197 mg/L
Alkalinity ($CaCO_3$)	143 mg/L
TSS	<5 mg/L
Ammonia (Distillation as N)	<1 mg/L
Phosphorus (Total as P)	<0.06 mg/L
Sulfate	60 mg/L
COD	<7 mg/L
Cyanide	<0.005 mg/L
Antimony	<0.04 mg/L
Arsenic	<0.01 mg/L
Barium	0.14 mg/L
Beryllium	<0.003 mg/L
Cadmium	<0.001 mg/L
Chromium	<0.002 mg/L
Copper	0.09 mg/L
Iron	<0.1 mg/L
Lead	<0.002 mg/L
Magnesium	18.3 mg/L
Manganese	<0.01 mg/L
Mercury	<0.0002 mg/L
Nickel	<0.05 mg/L
Fluoride	0.1 mg/L
Selenium	<0.004 mg/L
Silver	<0.002 mg/L
Zinc	<0.05 mg/L
TOC	<1 mg/L
NO_3-N	<2 mg/L
Thallium	<0.1 mg/L
Phenols	<0.005 mg/L
Lindane	<0.01 µg/L
Methoxychlor	<0.05 µg/L
Endrin	<0.01 µg/L
Toxaphene	<4 µg/L

TABLE 2: Length and Weight Measurements Taken During the Acute Toxicity Study of Whole Light Alkylate Product to Fathead Minnow[1]

Test Conc.	Rep.	Standard Length (mm) X	s	Weight (g) X*	s**	LF***
Control	A	16	1	0.06	0.02	0.14
Control	B	17	1	0.07	0.02	0.18
1.1 ppm	A	15	1	0.06	0.02	0.14
1.1 ppm	B	16	1	0.06	0.02	0.15
5.2 ppm	A	16	2	0.06	0.02	0.15
5.2 ppm	B	16	2	0.06	0.02	0.17
9.7 ppm	A	15	3	0.06	0.02	0.15
9.7 ppm	B	16	2	0.06	0.02	0.15
19 ppm	A	17	2	0.08	0.03	0.21
19 ppm	B	16	3	0.05	0.03	0.13
74 ppm	A	16	2	0.03	0.02	0.07
74 ppm	B	15	3	0.04	0.03	0.11

* X = Mean Value
** s = Standard Deviation
*** Loading Factor: g/Liter = $\frac{\text{Average Weight (g/fish) x No. fish in Test Chamber}}{\text{Test Chamber Vol. (Liters)}}$

[1] The pretest lengths and weights were, inadvertently, not measured.

TABLE 3: Acute Toxicity of Whole Light Alkylate Product to Fathead Minnow

	LC_{50}* (95% Confidence Limits)**			
	24 Hrs	48 Hrs	72 Hrs	96 Hrs
Nominal	19.6 ppm (5.2-74 ppm)	19.6 ppm (5.2-74 ppm)	8.2 ppm (5.2-9.7 ppm)	8.2 ppm (5.2-9.7 ppm)
Measured	553 ppb (254-1,206 ppb)	494 ppb (202-1,206 ppb)	323 ppb (183-398 ppb)	305 ppb (164-384 ppb)

* All LC_{50} values calculated using Binomial Probability Analysis.
** The 95% confidence limits presented above are not actually confidence limits because the LC50s were determined by binomial probability. The limits are statistically sound conservative bounds that are above 95% for the sample size used in this study.

	NOEC***			
	24 Hrs	48 Hrs	72 Hrs	96 Hrs
Nominal	19 ppm	5.2 ppm	5.2 ppm	5.2 ppm
Measured	622 ppb	204 ppb	184 ppb	166 ppb

*** All NOEC values calculated using Fisher's exact test.

TABLE 4: Cumulative Mortality During the Acute Toxicity Study of Whole Light Alkylate Product to Fathead Minnow

Exposure Time	Nominal Concentration (ppm)					
	Control	1.1	5.2	9.7	19	74
Day 0:						
1 hrs.	0/20	0/20	0/20	0/20	0/20	0/20
3 hrs.	0/20	0/20	0/20	0/20	0/20	0/20
6 hrs.	0/20	0/20	0/20	0/20	0/20	10/20
24 hrs.	0/20	0/20	0/20	14/20	2/20	20/20
Day 1:						
1 hrs.	0/20	0/20	0/20	14/20	2/20	20/20
3 hrs.	0/20	0/20	0/20	14/20	2/20	20/20
6 hrs.	0/20	0/20	0/20	14/20	2/20	20/20
24 hrs.	0/20	0/20	0/20	15/20	6/20	20/20
Day 2:						
1 hrs.	0/20	0/20	0/20	15/20	10/20	20/20
3 hrs.	0/20	0/20	0/20	15/20	11/20	20/20
6 hrs.	0/20	0/20	0/20	15/20	14/20	20/20
24 hrs.	0/20	0/20	0/20	15/20	19/20	20/20
Day 3:						
1 hrs.	0/20	0/20	0/20	15/20	20/20	20/20
3 hrs.	0/20	0/20	0/20	15/20	20/20	20/20
6 hrs.	0/20	0/20	0/20	15/20	20/20	20/20
24 hrs.	0/20	0/20	0/20	15/20	20/20	20/20

TABLE 5: Behavior Observations During the Acute Toxicity Study of Whole Light Alkylate Product To Fathead Minnow

Behavior of Survivors	Nominal Concentration (ppm)					
Exposure Time	Control	1.1	5.2	9.7	19	74
Day 0:						
1 hrs.	20A	20A	20A	20A	20A	17BV,3K
3 hrs.	20A	20A	20A	20A	20A	16BV,4K
6 hrs.	20A	20A	20A	20A	20A	5A,1O,4B
24 hrs.	20A	20A	20A	5A,1W	5A,13W	---
Day 1:						
1 hrs.	20A	20A	20A	5A,1W	5A,13W	---
3 hrs.	20A	20A	20A	5A,1W	5A,13W	---
6 hrs.	20A	20A	20A	5A,1WO	5A,13W	---
24 hrs.	20A	20A	20A	5A	13A,1B	---
Day 2:						
1 hrs.	20A	20A	20A	3A,2B	10A	---
3 hrs.	20A	20A	20A	3A,2B	4A,5B	---
6 hrs.	20A	20A	20A	3A,2B	1A,3JB,2JBV	---
24 hrs.	20A	20A	20A	3A,1B,1C	1C	---
Day 3:						
1 hrs.	20A	20A	20A	3BE,1B,1G	---	---
3 hrs.	20A	20A	20A	3BE,1B,1G	---	---
6 hrs.	20A	20A	20A	2BG,2G,1EG	---	---
24 hrs.	20A	20A	20A	5A	---	---

A - Normal
B - Quiescent
C - Hyperexcitable
E - Surfacing
G - Twitching
J - Ceased Swimming
K - Erratic Swimming
O - On side
V - Rapid Respiration
W - Slow Respiration

TABLE 6: Summary of Initial Water Quality Measurements Taken During the Acute Toxicity Study of Whole Light Alkylate Product to Fathead Minnow

Test Concentration	Temperature (°C) X	Temperature (°C) Range	pH Range	D.O. (ppm) X	D.O. (ppm) Range
Control	21.2	21.1-21.3	7.85-8.15	8.3	7.8-8.6
1.1 ppm	21.3	21.2-21.4	7.84-8.19	8.2	7.6-8.4
5.2 ppm	21.3	21.2-21.4	7.84-8.15	8.3	7.6-8.6
9.7 ppm	21.3	21.2-21.3	7.84-8.17	8.2	7.7-8.4
19 ppm	21.2	21.1-21.3	7.84-8.20	8.2	7.8-8.4
74 ppm	21.1	***	8.23 ***	8.3	***

* X = Mean Value
** s = Standard Deviation
*** Parameter only measured once during the study due to total mortality by 24 hours.

TABLE 7: Summary of Final Water Quality Measurements Taken During the Acute Toxicity Study of Whole Light Alkylate Product to Fathead Minnow

Test Conc.	Rep.	Temperature (°C) X	Temperature (°C) Range	pH Range	D.O. (ppm) X	D.O. (ppm) Range
Control	A	21.5	21.0-22.0	7.83-8.15	7.1	6.8-7.4
Control	B	21.6	21.1-22.1	7.82-8.12	7.1	6.9-7.3
1.1 ppm	A	21.6	21.2-22.1	7.83-8.15	7.2	7.0-7.6
1.1 ppm	B	21.6	21.2-22.1	7.87-8.17	7.2	7.0-7.4
5.2 ppm	A	21.5	21.0-22.1	7.66-8.14	7.4	7.2-7.8
5.2 ppm	B	21.5	21.0-22.0	7.87-8.16	7.4	7.1-7.8
9.7 ppm	A	21.6	21.1-22.2	7.87-8.17	7.4	7.1-7.6
9.7 ppm	B	21.6	21.1-22.2	7.91-8.09	7.4	7.2-7.6
19 ppm	A	21.7	21.2-22.2	7.92-8.08	7.4	7.2-7.6
19 ppm	B	21.6	21.1-22.3	7.89-8.06	7.2	7.1-7.3
74 ppm	A	***	***	***	***	***
74 ppm	B	***	***	***	***	***

* X = Mean Value
** s = Standard Deviation
*** Parameter not measured.

TABLE 8: Measured Exposure Concentrations During the Acute Toxicity Study of Whole Light Alkylate Product to Fathead Minnow

All values in ppm

Sample	0 hr. Initial	24 hr. Final	24 hr. Initial	48 hr. Final	48 hr. Initial	72 hr. Final	72 hr. Initial	96 hr. Final
Control	ND	0.016	ND	ND	0.006	ND	ND	ND
1.1 ppm	0.011	0.012	0.010	0.007	0.046	0.038	0.023	0.014
5.2 ppm	0.348	0.161	0.157	0.144	0.149	0.137	0.110	0.106
9.7 ppm	0.817	0.408	0.062	0.162	0.516	0.423	0.334	0.352
19 ppm	0.766	0.478	0.455	0.478	0.598	0.686	0.651	0.601
74 ppm	0.959	1.454	*	*	*	*	*	*

ND = Not detected at the method detetion limit.
* Only one initial and one final sample taken due to complete mortality at 24 hours.

TABLE 9a: Daily Cumulative Averages of the Measured Exposure Concentrations Determined for the Acute Toxicity Study of Whole Light Alkylate Product to Fathead Minnow

All values in ppm

Sample	24 hr. Avg.	48 hr. Avg.	72 hr. Avg.	96 hr (all samples) Avg.	Std. Dev.
Control	0.008	0.004	0.004	0.003	0.006
1.1 ppm	0.012	0.010	0.021	0.020	0.014
5.2 ppm	0.254	0.202	0.183	0.164	0.077
9.7 ppm	0.612	0.362	0.398	0.384	0.228
19 ppm	0.622	0.544	0.577	0.589	0.112
74 ppm *	1.206	1.206	1.206	1.206	0.350

TABLE 9b: Initial/Final Averages and Percent Retention of the Measured Exposure Concentrations Determined for the Acute Toxicity Study of Whole Light Alkylate Product to Fathead Minnow

All values in ppm

Sample	Average of Initial Samples	Average of Final Samples	Average % Retention
Control	0.002	0.004	>100
1.1 ppm	0.022	0.018	81.8
5.2 ppm	0.191	0.137	71.7
9.7 ppm	0.432	0.336	77.8
19 ppm	0.618	0.561	90.8
74 ppm *	0.959	1.454	>100

* Only one initial and one final sample taken due to complete mortality at 24 hours.

APPENDIX 1

STONYBROOK LABORATORIES INC.

To: J. F. Barbieri

Date: April 27, 1995

From: C.W. Chuang

CC: M.T. Benkinney
J.J. Yang

RE: ANALYSIS OF WHOLE LIGHT ALKYLATE PRODUCT IN WATER ACCOMMODATED FRACTION (WAF)

STUDY NO: 65908

The analysis of whole light alkylate product in WAF was performed following a purge-and-trap/gas chromatography procedure recently validated in-house (Study no. 65969). The results are revised as follows:

Table 1.1 Concentration of analytes in stock solutions prepared at 0 hour

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1100	0	ND*	ND	ND	ND	ND	ND	ND	----
2100	1.1	ND	ND	0.006	ND	ND	0.005	ND	0.011
3100	5.2	0.095	0.025	0.092	0.010	0.043	0.083	ND	0.348
4100	9.7	0.294	0.007	0.214	0.022	0.095	0.185	ND	0.817
5100	19	0.310	0.006	0.192	0.016	0.081	0.161	ND	0.766
6100	74	0.404	0.070	0.196	0.023	0.089	0.177	ND	0.959

* ND = not detected at the method detection limit (ref: Study no. 65969).

Table 1.2 Concentration of analytes of 24-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F24	0	0.005	ND	0.005	ND	ND	0.006	ND	0.016
2F24	1.1	0.003	ND	0.005	ND	ND	0.004	ND	0.012
3F24	5.2	0.059	0.012	0.037	ND	0.017	0.036	ND	0.161
4F24	9.7	0.153	0.033	0.088	0.011	0.040	0.079	0.004	0.408
5F24	19	0.205	0.036	0.095	0.010	0.042	0.085	0.005	0.478
6F24	74	0.611	0.106	0.305	0.039	0.140	0.253	ND	1.454

Table 2.1 Concentration of analytes in stock solutions prepared at 24 hours

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1I24	0	ND	ND	ND	ND	ND	ND	ND	----
2I24	1.1	0.005	ND	ND	ND	ND	0.005	ND	0.010
3I24	5.2	0.053	0.011	0.040	ND	0.018	0.035	ND	0.157
4I24	9.7	0.027	0.005	0.013	ND	0.006	0.011	ND	0.062
5I24	19	0.188	0.032	0.103	0.008	0.044	0.080	ND	0.455

Table 2.2 Concentration of analytes of 48-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F48	0	ND	ND	ND	ND	ND	ND	ND	----
2F48	1.1	0.004	ND	ND	ND	ND	0.003	ND	0.007
3F48	5.2	0.054	0.008	0.031	ND	0.015	0.036	ND	0.144
4F48	9.7	0.065	0.009	0.034	ND	0.017	0.037	ND	0.162
5F48	19	0.244	0.030	0.091	ND	0.035	0.076	0.002	0.478

Table 3.1 Concentration of analytes in stock solutions prepared at 48 hours

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1I48	0	ND	ND	ND	ND	ND	0.005	0.001	0.006
2I48	1.1	0.013	ND	0.014	ND	0.007	0.012	ND	0.046
3I48	5.2	0.042	0.010	0.037	ND	0.019	0.041	ND	0.149
4I48	9.7	0.129	0.034	0.135	0.013	0.068	0.135	0.002	0.516
5I48	19	0.214	0.043	0.134	0.014	0.060	0.129	0.004	0.598

Table 3.2 Concentration of analytes of 72-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F72	0	ND	ND	ND	ND	ND	ND	ND	----
2F72	1.1	0.014	ND	0.010	ND	0.005	0.009	ND	0.038
3F72	5.2	0.048	0.009	0.032	ND	0.015	0.033	ND	0.137
4F72	9.7	0.130	0.028	0.106	0.006	0.049	0.104	ND	0.423
5F72	19	0.303	0.049	0.144	0.010	0.057	0.121	0.002	0.686

Table 4.1 Concentration of analytes in stock solutions prepared at 72 hours

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1I72	0	ND	ND	ND	ND	ND	ND	ND	----
2I72	1.1	0.006	ND	0.006	ND	0.004	0.007	ND	0.023
3I72	5.2	0.035	0.009	0.023	0.005	0.013	0.022	0.003	0.110
4I72	9.7	0.151	0.024	0.072	0.006	0.027	0.054	ND	0.334
5I72	19	0.208	0.045	0.180	0.018	0.072	0.128	ND	0.651

Table 4.2 Concentration of analytes of 96-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F96	0	ND	ND	ND	ND	ND	ND	ND	----
2F96	1.1	0.008	ND	ND	ND	ND	0.006	ND	0.014
3F96	5.2	0.045	0.006	0.022	ND	0.010	0.023	ND	0.106
4F96	9.7	0.149	0.024	0.076	0.005	0.031	0.065	0.002	0.352
5F96	19	0.219	0.041	0.157	0.005	0.062	0.117	ND	0.601

Please call me to discuss the results.

APPENDIX 2

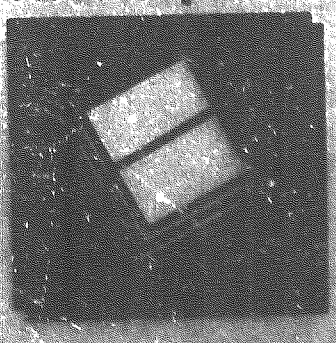
Stonybrook
Laboratories Inc.

Methods Validation for the Analysis of Whole Light Alkylate Product in Water Accommodated Fraction (WAF) Using Purge-and-Trap and GC/FID

Stonybrook Laboratories Inc.
Princeton, NJ

Study Number: 65869

Final Report



STONYBROOK LABORATORIES INC.

REPORT RELEASE

LIAISON: C.A. SCHREINER

STUDY NUMBER: 65969

CRU NUMBER: 24194

TEST ARTICLE: WHOLE LIGHT ALKYLATE PRODUCT

STUDY TITLE: METHODS VALIDATION FOR THE ANALYSIS OF WHOLE LIGHT ALKYLATE PRODUCT IN WATER ACCOMMODATED FRACTION (WAF) USING PURGE-AND-TRAP AND GC/FID

RESULTS:

The development and validation of a purge-and-trap/gas chromatography (PT/GC) method for the analysis of water acclimated fractions (WAF) of whole light alkylate product and the subsequent determination of optimal WAF equilibration times has been completed. The method was developed and validated using seven C6-C8 alkane and cycloalkane standards which represent 68% of the whole light alkylate product. The sensitivity and precision of the assay were validated at the 5 part-per-billion (PPB) level for each of the seven component standards in water. Using this technique, it was determined that the whole light alkylate product freshwater WAF reached equilibrium in approximately 24 hours at a total WAF concentration (sum of n=7 components) of 1.6 parts-per-million (PPM). The saltwater WAF reached equilibrium in approximately 12 hours at a total concentration (sum of n=7 components) of 0.9 PPM.

T.A. Roy 1/30/95
T.A. Roy Date
Study Director

C.A. Schreiner 1/30/95
C.A. Schreiner Date
Vice-President

C.R. Mackerer 2/1/95
C.R. Mackerer
President Date

DISTRIBUTION:
All above, Liaison/C.A. Schreiner, Archives
STUDY NO. 65969

STATEMENT OF COMPLIANCE

The undersigned hereby state that Study No. 65969, Methods Validation for the Analysis of Whole Light Alkylate Product in Water Accommodatated Fraction (WAF) Using Perge-and-Trap and GC/FID, was conducted in compliance with the Good Laboratory Practice Regulations as published in 40 CFR Part 792 Federal Registrar Volume 54-158, 8/17/89 in all aspects with the following exceptions:

The strength, purity and composition or other characteristics to define the test substance was not determined by the testing facility. The methods of synthesis, fabrication, or derivation of the test substance are the responsibility of the sponsor and the data are located at the sponsor's facility.

The purity of purchased reference materials was not determined by the testing facility. It is not known if the purity determination of these chemicals by the supplier were performed under GLPs.

The data acquisition or analysis software on the HP MS DOS operating system used in the study has not been validated in-house.

No bulk inventory usage log was mantained for the test chemicals or analytical standards.

T. A. Roy
T. A. Roy
Study Director

Gary A. Ramsina
G. A. Ramsina
Study Sponsor

TABLE OF CONTENTS

SUMMARY

The development and validation of a purge-and-trap/gas chromatography (PT/GC) method for the analysis of water acclimated fractions (WAF) of whole light alkylate product and the subsequent determination of optimal WAF equilibration times has been completed. The method was developed and validated using seven C6-C8 alkane and cycloalkane standards which represent 68% of the whole light alkylate product. The sensitivity and precision of the assay were validated at the 5 part-per-billion (PPB) level for each of the seven component standards in water. Using this technique, it was determined that the whole light alkylate product freshwater WAF reached equilibrium in approximately 24 hours at a total WAF concentration (sum of n=7 components) of 1.6 parts-per-million (PPM). The saltwater WAF reached equilibrium in approximately 12 hours at a total concentration (sum of n=7 components) of 0.9 PPM.

EXPERIMENTAL

EXPERIMENTAL DESIGN SUMMARY:

Seven C6-C8 alkanes and cycloalkane, which represent 68% of whole light alkylate product, were selected as the monitored analytes for the in-house method validation. The analyte in methanol solution was spiked into 5 mL deionized water. The aqueous solution were loaded into the purge-and-trap sparger by a Luer Lock syringe. The analytes were then purged out by helium from the aqueous phase to the vapor phase at ambient temperature. The vapor was transferred and consequently trapped in a sorbent tube. After the purge was completed, the sorbent tube was then backflushed and heated. The analytes were swept by helium onto the head of the GC column where the separation and detection took place. The evaluations included measuring each compound's response sensitivity, reproducibility, and purge efficiency. Once the analytical procedure had been verified, a WAF of Whole Light Alkylate Product was generated and evaluated at different time intervals to demonstrate the suitability of the proposed WAF generation procedure.

TEST SUBSTANCES:

ANALYTE NAME	CRU #	LOT #	EXPIRATION	PURITY
2-methylbutane (isopentane)	94570	03859DG	9/99	99%
2,3-dimethylbutane	94565	LA-44304	9/99	99%
2,4-dimethylpentane	94565	LA-44304	9/99	99%
2,5-dimethylhexane	94565	LA-44304	9/99	99%
2,2,4-trimethylpentane	94565	LA-44304	9/99	99%
2,3,4-trimethylpentane	94565	LA-44304	9/99	99%
hexane (surrogate)	110-54-3*	42H06471	1/99	99%
2,3,3-trimethylpentane	94591	244X-5S	10/99	99%
1-methyl-ethylcyclopentane	94590	2360	10/99	99%

*CAS Number

Chemical purity and stability data for reference standards purchased commercially were provided by the suppliers (Supelco, Sigma, Wiley, API Standard Reference Materials). The data provided by the suppliers is archived with the raw data.

APPARATUS AND REAGENTS:

Syringe--5 mL gas-tight glass with Luer Lock.
Micro syringes--10 µL, 25 µL, 50 µL, 100 µL, and 250 µL.
GC vials--Glass with Teflon-lined screw caps.
Volumetric flasks--Variable volume size with ground-glass stoppers.
Analytical balance--0.0001 g.
Methanol--HPLC grade.

Secondary working standard mixes--Two standard mixes of the eight whole light alkylate component alkanes plus hexane were prepared by mixing their individual stock standard in methanol for a concentration of 100 µg/mL: mix I: isopentane, 2,3, 3-trimethypentane, and 1-methyl-1-ethylcyclopentane and mix II contained the remaining 5 analytes plus the surrogate, hexane.

Calibration standards--Five levels of standards (approximately 1, 5, 10, 25 and 50 µg/mL) were prepared from the secondary working standard mixes.

Spiking surrogate standard--An approximately 10 µg/mL of hexane was prepared in methanol from the stock standard. This solution was spiked in all blanks, spikes, and samples prior to analysis.

Storage and handling precautions --All solutions (except stock standards) were stored at 4°C and labeled with study number, names, concentrations, and expiration date. All solutions will be disposed of upon release of the final report

PROCEDURE:

Set up the acquisition sequence on the Waters chromatography data system.

A 5 mL Luer Lock syringe is filled to overflowing with deionized water which has also been heated to boiling to remove residual volatile organics. The plunger is replaced and the water compressed to the 5 mL mark. The plunger is pulled back slightly to allow for the addition of 5 µL of calibration standard or spiking surrogate standard. After the solution is loaded to the P&T, press START on the LSC 2000 front panel to start the purge-and-trap procedure.

Initial calibration - Run five levels of calibration standards following the procedure described above and calculate the response factor (RF) of the individual analytes based on equation (I):

$$RF = A_S/C_S \qquad (I)$$

where:

A_S: peak area count of analyte

C_S: amount in nanograms (e.g., 5 µL of a 1.0 µg/mL solution = 5 ng) of the calibration standard injected into the syringe

Calculate the average response factor (RF_{ave}) and standard deviation (SD) of five-level calibration standards. Calculate the relative standard deviation (%RSD = (SD/RF_{ave}) x 100) of the calibration using Microsoft Excel (version 4.0). If %RSD is < 20%, then the RF_{ave} of the analytes is used for quantitation. If %RSD > 20%, the first degree linear regression (forced through zero) with r> 0.99 is used for quantitation (re: quantitative analysis section).

Sample analysis - The analysis follows the steps described above. Samples were analyzed only once using one of two duplicate sample vials except when a need for further confirmation arose or when dilutions were required to bring the response of the analytes within the range of the calibration standards. The duplicate sampling vials were used in these cases.

WAF GENERATION AND EVALUATION:

Two types of WAFs of Whole Light Alkylate Product were evaluated to demonstrate equilibrium and maintenance of test material. A WAF prepared with freshwater was evaluated at 0,1,3,6,24,36,48,60 and 72 hours after preparation while a WAF prepared with saltwater was evaluated at 0,1,3,6,12,24,36 and 48 hours after preparation. The WAFs were generated following modification of the procedure used by Anderson, et al (1974, Marine Biol., 27: 75-88). Two WAFs were prepared, using each water type, containing 50 ppm of Whole Light Alkylate Product. One WAF of each water type was prepared in a bottle filled to the neck to minimize headspace ("XXX1X" sample designation, e.g, sample "3FW2B" is a 3-hour, freshwater, type 1 WAF, the second of duplicate samples collected), while the second WAF of each water type was prepared in a bottle filled to the shoulder to maximize product-water contact ("XXX2X" sample designation). Duplicate samples were collected from each bottle (except for time zero "XXX2X" series) at the specified time periods, with one sample analyzed using the methodology determined from the in-house validation and the other sample acting as a backup. All samples were collected in 40 ml glass vials with no headspace. The concentration in each flask was quantified to evaluate the consistency of the WAF with time, water type and stirring procedure.

GOOD LABORATORY PRACTICES:

This study was conducted according to the EPA Good Laboratory Practice Standards outlined in 40 CFR Part 160, Federal Register Vol. 54, No.158, 8/17/89.

Test Substance(s) Characterization - The methods of synthesis, fabrication, and/or derivation of the test materials is the responsibility of the sponsor. In addition, the stability, identity, strength, purity and composition of other characteristics which identify the test materials are the responsibility of the sponsor. The test article data are located at the sponsor's facility.

Chemical purity and stability data for reference and control standards purchased commercially, with the exception of 2,3,3-trimethylpentane and 1-methyl-ethylcyclopentane, were provided by the suppliers (Supelco, Sigma). The latter two compounds were assayed for purity at Stonybrook Laboratories Inc. These data and those provided by the suppliers are archived with the raw data.

RECORDS MAINTAINED:

The study file contains but is not limited to the following records or verified copies of:

Notice of Intent to Initiate Study
Request for Testing
Sponsor Protocol Amendment Approval Memo
Study Protocol and Amendments
Technical Personnel Records
Reagents and Equipment Inventory
Chemical Repository Unit (CRU) Dispensing Records
Study Notebook Records

RESULTS & DISCUSSION

METHOD EVALUATION/VALIDATION:

The use of the PT/GC technique for the analysis of whole light alkylate product WAF was based on a review of the test article composition and the anticipated composition of the WAF. The use of PT/GC runs throughout the EPA analytical methods series for drinking water (500), municipal/industrial effluent water (600) and wastewater (8000). The method has been tentatively validated for the analysis of gasoline range organics (GRO) in the last year and drafts of the method were made available by the Office of Solid Waste (OSW) prior to the expected promulgation in late 1994.

Six alkanes and one cycloalkane were selected (representing 68% of the components of the test material) for the in-house evaluation/validation. Hexane was chosen as the surrogate. The EPA procedure for the evaluation of method performance is an appropriate standard by which to assess in-house method validation. Determination of the method detection limit (MDL), limit of detection (LOD) and limit of quantitation (LOQ) provide an excellent measure of the sensitivity and precision of the procedure. MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The LOD is the lowest concentration that can be determined statistically differently from the blank. LOD is numerically defined as three times the standard deviation from replicate measurements of standard. LOQ is the level above which quantitative results may be obtained and is numerically defined as ten times the standard deviation from replicate measurements of standards. The LOD, LOQ and MDL were determined from replicate measurements of the analytes and surrogate in water at 5 PPB. In general, the per component MDL was slightly below 5 PPB. The LOD, LOQ and MDL for each of the compounds is reported in table I.

WAF GENERATION AND EVALUATION:

Two types of WAF were generated to evaluate the affect of mixing and headspace on final WAF concentration. The concentration of test article components was significantly higher (factor of 2) in the "minimal headspace" type WAF as compared to the "maximum phase interface" type WAF. Table II reports the time course of WAF concentration for the individual and summed seven analytes monitored for both freshwater (through 72 hours) and saltwater (through 48 hours). The surrogate recoveries, which were essentially quantitatve, are also reported for each WAF sample analyzed.

WAF concentration of test material peaked at approximately 12 hours in saltwater (0.9 PPM) and 24 hours in freshwater (1.6 PPM) using the "minimal headspace"WAF generation procedure. This can be seen more clearly in Figure 1 where the "Total" column data in table II for freshwater and saltwater WAF concentrations are plotted vs time of sampling in a histogram format. Figure 2 plots the individual component concentrations for freshwater and saltwater WAF vs sampling time and shows that the relative concentration of the individual test article WAF components is largely maintained over the mixing period. Figures 3 and 4 compare the 24 hour WAF concentration of the test article components with the actual concentration of the components in the test article. These experimentally observed results can be predicted with a reasonable degree of accuracy if the water solubility or octanol/water partition coefficients of the components are taken into consideration.

Table 1

Summary Sheet for LOD, LOQ and MDL Determinations for Whole Light Alkylate Product WAF Components and Surrogate

Peak#	Compound	Rt. (min.)	Area counts						
			Run1	Run2	Run3	Run4	Run5	Run6	Run7
1	2,3-dimethylbutane	8.865	37422	31806	36712	34842	30650	20817	46749
2	hexane (surr)	8.850	35686	30139	34788	34045	28001	19625	46398
3	2,4-dimethylpentane	9.600	41090	34678	40270	37909	33107	22357	52058
4	2,2,4-trimethylpentane	11.420	43558	36274	41736	41538	36182	25682	54862
5	2,5-dimethylhexane	12.750	40754	34303	39308	40101	34222	24153	51361
6	2,3,4-trimethylpentane	13.480	41512	35834	41050	42265	37296	25760	53336
7	2,3,3-trimethylpentane	13.600	41454	36507	41116	42461	38096	35948	41716
8	1-methyl-1-ethylcyclopentane	15.115	46856	42044	46061	49500	44715	45595	47700

Peak#	Compound	Rt. (min.)	FFSCL ODLOQ R1	FFSCL ODLO QR2	FFSCL ODLO QR3	FFSCL ODLOQ R4	FFSCL ODLOQ R5	FFSCL ODLO QR6	FFSCL ODLO QR7	RF(ave)	Std. Dev.	%RSD	Std. Dev. (ppb)	LOD (ppb)	LOQ (ppb)	t value*	MDL (ppb) =Std. Dev. x t
			Response factor=Area count/5 (ng)														
1	2,3-dimethylbutane	8.865	7484.4	6379.2	7342.4	6968.4	6130.0	4163.4	9349.8	6831.1	1573.9	23.0	1.2	3.5	12	3.71	4.3
2	hexane (surr)	8.850	7139.2	6031.8	6957.6	6809.0	5600.2	3925.0	9279.6	6534.6	1637.6	25.1	1.3	3.8	13	3.71	4.6
3	2,4-dimethylpentane	9.600	8219.6	6935.6	8054.0	7581.8	6623.4	4471.4	10411.6	7470.8	1805.8	24.2	1.2	3.6	12	3.71	4.5
4	2,2,4-trimethylpentane	11.420	8711.6	7254.8	8347.2	8307.6	7236.4	5136.4	10972.4	7995.2	1774.6	22.2	1.1	3.3	11	3.71	4.1
5	2,5-dimethylhexane	12.750	8150.8	6860.6	7861.6	8020.2	6844.4	4830.6	10272.2	7548.6	1656.2	21.9	1.1	3.3	11	3.71	4.1
6	2,3,4-trimethylpentane	13.480	8302.4	7166.8	8210.0	8453.0	7439.2	5152.0	10667.2	7915.8	1638.5	21.0	1.0	3.1	10	3.71	3.9
7	2,3,3-trimethylpentane	13.600	8290.8	7301.4	8223.2	8492.2	7619.2	7189.6	8343.2	7922.8	538.9	6.80	0.34	1.0	3.4	3.71	1.3
8	1-methyl-1-ethylcyclopentane	15.115	9371.2	8408.8	9212.2	9500.0	8943.0	9119.0	9540.0	9213.5	471.2	5.11	0.26	0.77	2.6	3.71	0.95

* t value at 99% confidence interval

Table II
Continued

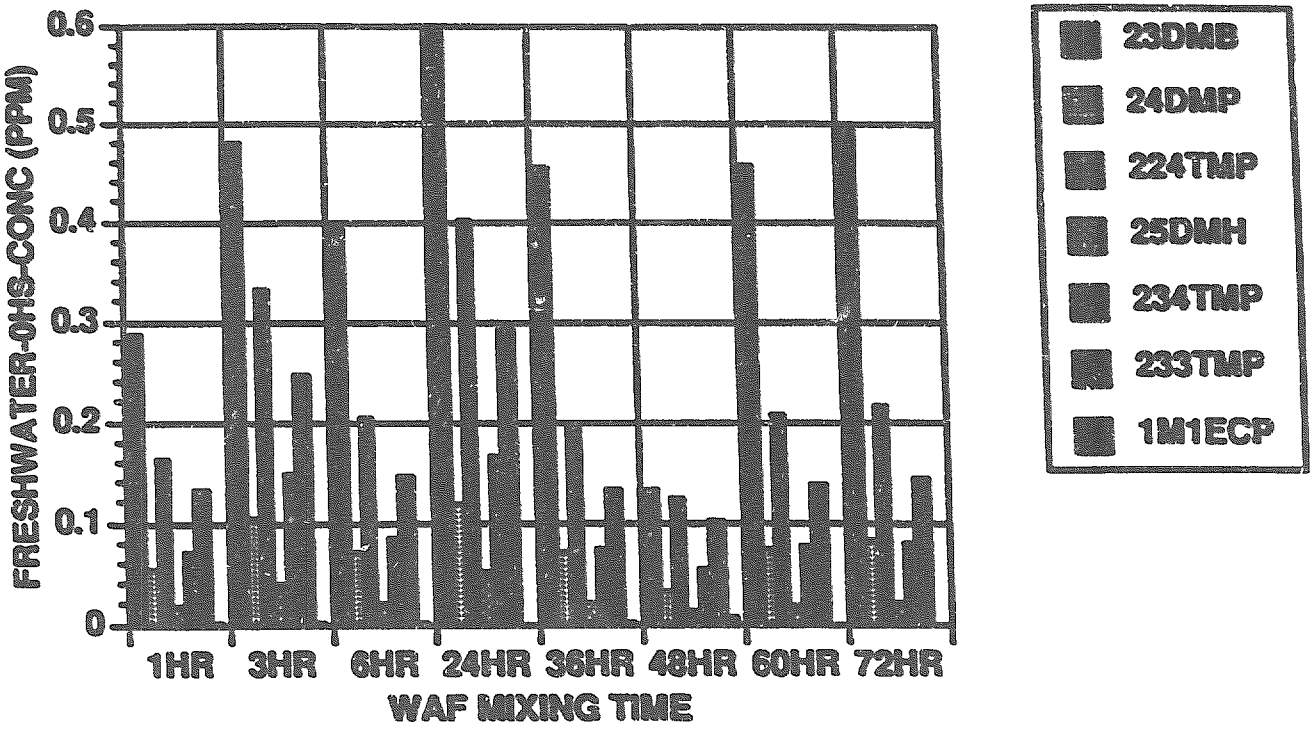
		2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total	hexane (surr) recovery (%)
	RF(ave)	*7524.2*	*8025.4*	*8569.9*	*8495.5*	*8756.4*	*8818.6*	*9204.0*		*7378.6*
Data file	DF*									
36FW1A	20	0.455	0.072	0.197	0.022	0.073	0.133	0.002	0.953	111
36FW1A	10	0.372	0.056	0.145	0.016	0.055	0.101	0.002	0.747	114
36FW1A	10	0.372	0.056	0.145	0.014	0.055	0.103	0.000	0.547	109
36FW2A	20	0.206	0.041	0.128	0.014	0.052	0.100	0.000	0.492	102
36FW2A	10	0.174	0.036	0.117	0.014	0.052	0.100	0.000	0.465	110
48FW1A	20	0.132	0.031	0.125	0.016	0.054	0.102	0.006	0.672	109
48FW1A	10	0.327	0.049	0.134	0.013	0.050	0.099	0.000	0.812	81
48FW2A	20	0.331	0.062	0.186	0.019	0.074	0.140	0.000	0.438	114
48FW2A	10	0.166	0.033	0.100	0.012	0.044	0.084	0.000	0.972	102
60FW1A	20	0.456	0.074	0.206	0.021	0.076	0.138	0.000	0.733	109
60FW2A	20	0.242	0.055	0.184	0.024	0.082	0.146	0.000	1.042	109
72FW1A	20	0.500	0.081	0.216	0.023	0.079	0.143	0.000	0.595	101
72FW2A	20	0.222	0.046	0.142	0.016	0.059	0.110	0.000		

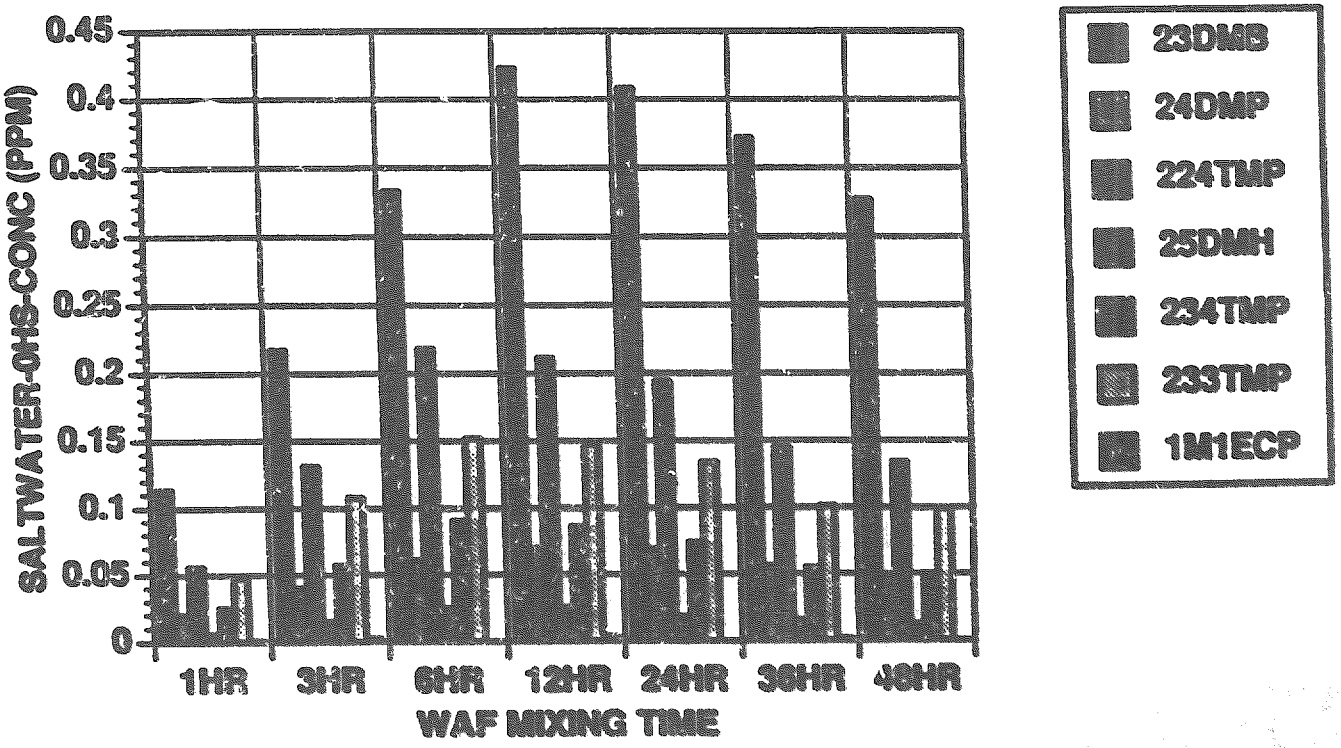
*dilution factor

Data file format - e.g., 36FW1A = 36 hour collection time, freshwater, type "1" WAF (see experimental section), "A", first of two (duplicate) samples collected at the indicated time point.

Figure 2

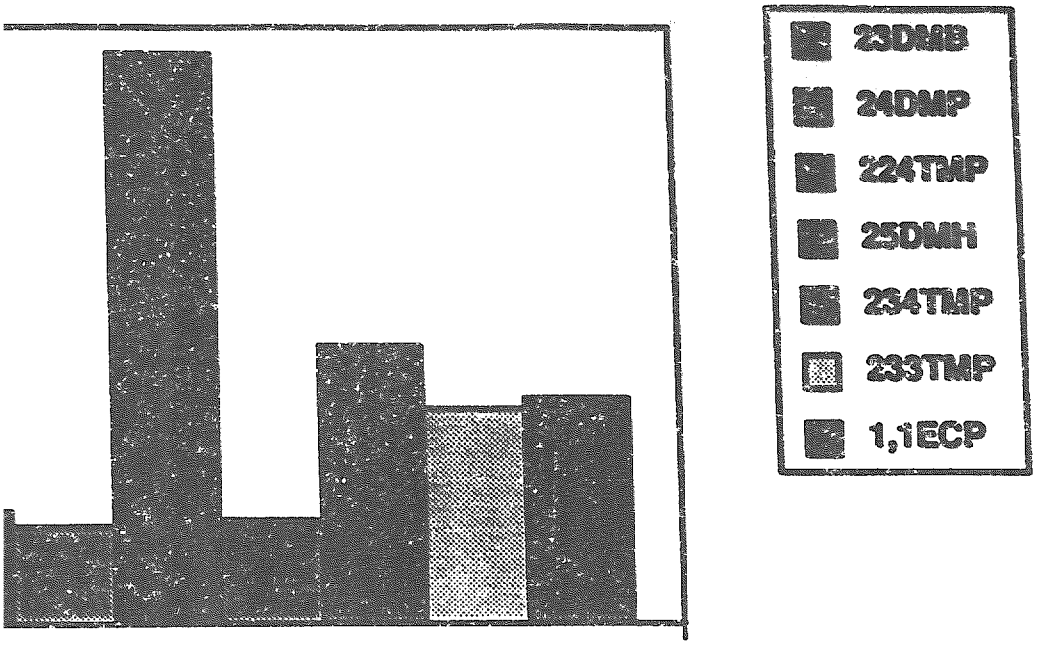
Individual Monitored Component Concentrations in Whole Light Alkylate Product Freshwater and Saltwater WAFs over 48-72 Hours

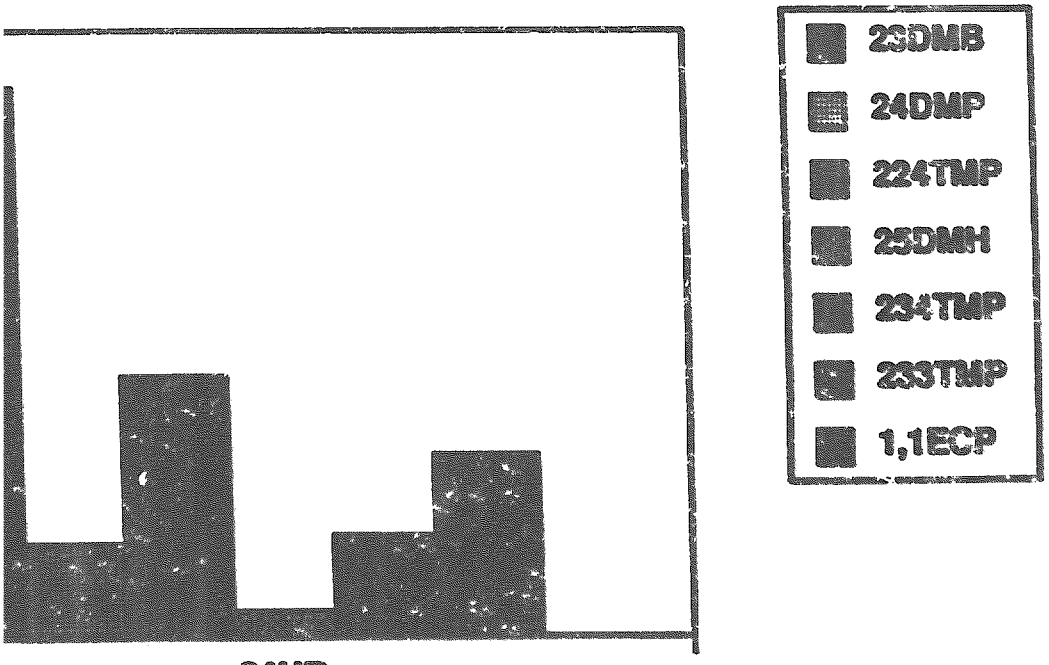




of Whole Light Alkylate Product Alkane Concentrations In
terial With Their 24 Hour WAF Concentration (Saltwater)

969 10/26/94 DATA





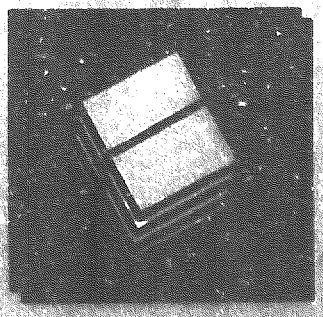
24HR

Stonybrook
Laboratories Inc.

Static-Renewal 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to Silverside Minnow

Stonybrook Laboratories Inc.
Princeton, NJ

Final Report



Study Number 65911

STONYBROOK LABORATORIES INC.
REPORT RELEASE

TO STUDY DIRECTOR/LIAISON: C.A. Schreiner
STUDY NUMBER: 65911
CRU NUMBER: 94194
SAMPLE NAME: Whole Light Alkylate Product
STUDY TITLE: Static-Renewal 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to Silverside Minnow
REQUESTER: Petroleum Product Stewardship Council

RESULTS:

LC_{50} 27 ppm for Whole Light Alkylate Product (nominal)
LC_{50} 423 ppb for Whole Light Alkylate Product (measured)

A static-renewal 96-hour toxicity study was conducted December 12-16, 1994 to determine the acute toxicity of Whole Light Alkylate Product to silverside minnow, a representative salt water fish species. Test fish were exposed to individual water accommodated fractions (WAFs) of the poorly water-soluble test material at nominal concentrations of 3 ppm, 12 ppm, 22 ppm, 52 ppm, and 97 ppm (w/v, based on density). Nominal test concentrations are based on the loading rate, or amount of test material added to make each WAF. Test solutions were renewed at 24 hour intervals during conduct of the study. Water quality parameters of pH, temperature, salinity, and dissolved oxygen (D.O.) were measured throughout the study.

Samples of the control and exposure concentrations were collected daily and quantitatively analyzed using purge-and-trap/gas chromatography (GC). The concentrations were quantitated using Whole Light Alkylate Product component standards. Measured test concentrations are based on the total concentration of all analytes. Test material retention from the static-renewal procedure ranged from 57.1-90.1%, producing consistent exposure of the test fish to Whole Light Alkylate Product throughout the study.

The toxicity of the test material was evaluated on the basis of LC_{50} determinations at 24, 48, 72, and 96 hours. The term LC_{50} used in this report refers to the concentration causing 50% mortality after a specified exposure period. The computer-estimated 96-hour LC_{50} for Whole Light Alkylate Product to silverside minnow under static-renewal test conditions was 27 ppm based on nominal exposure concentrations, and 423 ppb based on measured exposure concentrations. The 96-hour no observed effect concentration (NOEC), based on nominal concentrations, was 12 ppm, since exposure to concentrations of 22 ppm and greater resulted in significant mortality. The 96-hour no observed effect concentration (NOEC), based on measured concentrations, was 160 ppb, since exposure to concentrations of 306 ppb and greater resulted in significant mortality.

Approvals:

J.F. Barbieri/MTB 12/1/95
Study Director/Date
J.F. Barbieri

M.T. BenKinney 12/1/95
Supervisor/Date
M.T. BenKinney

C.R. Mackerer 12/1/95
President/Date
C.R. Mackerer

Distribution: Study Director, Liaison, Archives (Original)

STATIC-RENEWAL 96-HOUR ACUTE TOXICITY STUDY OF THE WATER ACCOMMODATED FRACTION (WAF) OF WHOLE LIGHT ALKYLATE PRODUCT TO SILVERSIDE MINNOW

STUDY No.: 65911

MATERIAL TESTED:	Whole Light Alkylate Product
CRU SAMPLE No.:	94194
REQUESTER:	Petroleum Product Stewardship Council c/o Synthetic Organic Chemical Manufacturing Association 1100 NY Ave., NW, Suite 1090 Washington, D.C. 20005
STUDY PERFORMED BY:	Stonybrook Laboratories Inc. 311 Pennington-Rocky Hill Road Pennington, N.J. 08534
STUDY INITIATION DATE:	July 22, 1994
EXPERIMENTAL START DATE:	November 21, 1994
EXPERIMENTAL TERMINATION DATE:	January 4, 1995

Compliance Statement

Study No. 65911

This study was conducted according to the USEPA Toxic Substances Control; Good Laboratory Practice Standards. 40 CFR Part 792, except as noted below; the final report fully and accurately reflects the raw data generated in the study.

Exceptions to GLPs:

1. The test material, Whole Light Alkylate Product, was not characterized and stability analysis was not performed at this facility.

2. Some data entries were made late. These late entries were indicated as such.

3. Some equipment logs were not up to date at the time of the study.

J. F. Barlow/MBK — 12/1/95

Study Director — Date

STONYBROOK LABORATORIES INC.

QUALITY ASSURANCE STATEMENT

Study Number: 65911

Title of Study: Static-Renewal 96-Hour Acute Toxicity Study of the Water Accommodated Fraction (WAF) of Whole Light Alkylate Product to Silverside Minnow

Listed below are the dates that this study was reviewed by the Quality Assurance Unit and the dates that the findings were reviewed by the Study Director and Management.

DATE(S) OF QA REVIEW	PHASE OF STUDY	DATE(S) REVIEWED BY STUDY DIRECTOR	DATE(S) REVIEWED BY MANAGEMENT
11/18/94	PROTOCOL REVIEW	2/3/95	2/25/95
12/12/94	IN-PROCESS INSPECTION	2/19/95	2/25/95
4/11/95	FINAL REPORT AUDIT	5/12/95	7/18/95

Lisa Mick
Manager, Quality Assurance

7/26/95
Date

J.S. Gross* / MTB
PRINCIPAL INVESTIGATOR

J.F. Barbieri* / MTB
STUDY DIRECTOR 12/1/85

* no longer with company
MTB 12/1/85

DISTRIBUTION:

Liaison:	C.A. Schreiner, Ph.D.
Principal Investigator:	J.S. Gross, B.S.
Study Director:	J.F. Barbieri, B.S.
Supervisor:	M.T. BenKinney, M.S.
President, Stonybrook Laboratories Inc.:	C.R. Mackerer, Ph.D.
Archives	

OTHER PERSONNEL INVOLVED IN THE STUDY

A. L. McClurg : Laboratory Technician

N.L. Afonina : Laboratory Technician

C.W. Chuang : Study Chemist

A.L. Crawford : Culturist

A.L. Wagstaff : Laboratory Technician

TABLE OF CONTENTS

LIST OF TABLES

SUMMARY:

A static-renewal 96-hour toxicity study was conducted December 12-16, 1994 to determine the acute toxicity of Whole Light Alkylate Product to silverside minnow, a representative salt water fish species. Test fish were exposed to individual water accommodated fractions (WAFs)of the poorly water-soluble test material at nominal concentrations of 3 ppm, 12 ppm, 22 ppm, 52 ppm, and 97 ppm (w/v, based on density). Nominal test concentrations are based on the loading rate, or amount of test material added to make each WAF. Test solutions were renewed at 24 hour intervals during conduct of the study. Water quality parameters of pH, temperature, salinity, and dissolved oxygen (D.O.) were measured throughout the study.

Samples of the control and exposure concentrations were collected daily and quantitatively analyzed using purge-and-trap/gas chromatography (GC). The concentrations were quantitated using Whole Light Alkylate Product component standards. Measured test concentrations are based on the total concentration of all analytes. Test material retention from the static-renewal procedure ranged from 57.1-90.1%, producing consistent exposure of the test fish to Whole Light Alkylate Product throughout the study.

The toxicity of the test material was evaluated on the basis of LC_{50} determinations at 24, 48, 72, and 96 hours. The term LC_{50} used in this report refers to the concentration causing 50% mortality after a specified exposure period. The computer-estimated 96-hour LC_{50} for Whole Light Alkylate Product to silverside minnow under static-renewal test conditions was 27 ppm based on nominal exposure concentrations, and 423 ppb based on measured exposure concentrations. The 96-hour no observed effect concentration (NOEC), based on nominal concentrations, was 12 ppm, since exposure to concentrations of 22 ppm and greater resulted in significant mortality. The 96-hour no observed effect concentration (NOEC), based on measured concentrations, was 160 ppb, since exposure to concentrations of 306 ppb and greater resulted in significant mortality.

INTRODUCTION:

The objective of this study was to determine the acute toxicity of Whole Light Alkylate Product to aquatic organisms by evaluating its effect on silverside minnow (*Menidia beryllina*), a representative salt water fish species. Silverside minnow were selected since they are a salt water test species recommended in U.S. EPA (1) regulations. Static-renewal testing of the water accommodated fraction (WAF) in closed containers with no headspace was chosen as the most appropriate study design for the test material, due to the volatile nature of the test material. Under WAF exposure conditions, toxic effects from the soluble components of the test material are evaluated.

The analytical standards chosen to evaluate the WAF of Whole Light Alkylate Product were selected as representative of the alkane and cycloalkane constituents which account for 68% of the test material. These constituents were expected to be found in the highest concentrations in the WAF and account for most, if not all, of the toxicity measured during the study.

In acute toxicity tests, the most commonly used adverse effect criterion is death of the organism. Mortality data collected during the study are used to calculate an LC_{50} (concentration lethal to 50% of the test population after a specific time period which is typically 96 hours).

METHODS AND MATERIALS:

Test Fish:

The juvenile silverside minnow (*Menidia beryllina)* used in the study were purchased from ARO, inc, Hampton, NH. The fish were acclimated in-house in a tank filled with Mobil Technical Center (MTC) well water (Table 1) that had been salinity adjusted with Forty Fathoms Synthetic Seawater Mix. Acclimation prior to experimentation lasted a minimum of 14 days for the definitive study on a 16-hour light/8-hour dark cycle (fluorescent lighting) following acceptable culturing techniques (2,3,4,5). The fish were fed a commercial fish food (Wardley's flake) and Artemia sp. nauplii (24±6 hrs. old) *ad libitum* during acclimation. Temperature in the holding tank was maintained at 20 ± 2 °C during the acclimation period and mortality of the test population was <5% in the 48 hours prior to study initiation. The silverside minnow were not fed for 21.5 hours preceding the definitive study initiation nor during conduct of the study. Since individual identification of the fish was not possible, silverside minnow were netted and arbitrarily added to each test chamber. The loading factor of the test population (Table 2) was determined to be less than 1.0 g/L. All intact fish collected in the study were weighed and measured after test termination (Table 2).

Test System:

The Whole Light Alkylate Product static-renewal toxicity study was conducted in labeled 3.8 liter glass jars, sealed with teflon lined screw caps. The test jar labeling included the study number, CRU number, test date, concentration, group number, replicate letter, and species designation. Test jars contained 3.8 liters of test solution, allowing no headspace. The water source for the study was MTC well water adjusted to a salinity of 20±2 ppt. The test exposure chambers were held in a water bath maintained at 20 ± 1 °C for the first day of the study, then were moved to an incubator for the remainder of the study, at the same temperature. The photoperiod during testing was 16-hr light/8-hr dark (fluorescent lighting).

The silverside minnow were exposed to individual WAF solutions of Whole Light Alkylate Product. Generation of the WAF solutions was produced following a modification of the procedure used by Anderson, et al., 1974 (6). Approximately fourteen hours prior to test initiation, six individual WAF 9 liter bottles were set up. A stir bar and 9.4 liters of test water were placed into each bottle. A 9 liter bottle filled to the neck (instead of the normal shoulder height) can hold 9.4 liters. The bottles were filled to neck height to minimize volatility. A measured amount of Whole Light Alkylate Product (nominal concentration), calculated for each exposure concentration, was added to each bottle. All bottles were capped tightly with a positive pressure siphoning apparatus and parafilm and covered with aluminum foil. The siphoning apparatus was comprised of a teflon lined rubber stopper housing two teflon tubes. One long siphon tube extended to the bottom of the WAF solution. The other tube ended above the WAF surface, and was used to control air pressure while siphoning. The teflon tubes were glass stoppered until use. The stirring speed of the bottles was adjusted to produce a vortex of less than 25% of the container depth. The solutions stirred for approximately 12 hours, and then were allowed to settle for approximately 45 minutes. After the stirring/settling period, the aqueous phase (WAF) was siphoned using positive air pressure. Two 3.8 liter replicates were prepared from each individual WAF. A sample was also collected for initial water quality measurements. Forty ml samples were also taken of the WAF for chemical analysis. The solution in each test container was renewed daily during the study. The renewal concentrations were produced in the same manner as the initial concentrations. The test fish remained in the test container during the renewal process.

Test Material:

The test material, Whole Light Alkylate Product, was dispensed by Stonybrook Laboratory's Chemical Repository Unit (CRU) from a homogeneous sample obtained from the sponsor. As reported in the Product Physical and Chemical Data (PPCD) sheet, Whole Light Alkylate Product (CRU No. 94194) consists entirely of Light Alkylate Naphtha. It was received as a liquid. The stability, identity, strength, purity, and composition or other characteristics which identified the test material was the responsibility of the sponsor. The concentrations used in this study were prepared by pipetting known quantities into each WAF bottle on a weight to volume basis, based on the density (0.7 g/ml) of the test material. Following a stirring and settling period, the aqueous phase of each solution was used for its corresponding exposure concentration.

Test Procedure-Biological:

A preliminary test was performed November 21-23, 1994, to assess the toxicity of the test material under closed container static renewal conditions. This range finding study consisted of 10 fish per replicate exposed to a control and three concentrations of 0.97 ppm, 9.7 ppm, and 97 ppm, evaluated in duplicate. At test termination, no mortality was observed in the 0.97 ppm or 9.7 ppm concentrations, with insignificant mortality (1 fish, 5%) in the control. Also at 48 hours, total mortality was observed in the 97 ppm concentration. Based on these results, a dose range of 3-97 ppm was chosen for the definitive study.

The 96-hour definitive toxicity study documented in this report was conducted December 12-16, 1994. This study was conducted using a static-renewal test procedure, with daily replacement of solution in each test chamber. All concentrations were run in duplicate in 3.8 liter glass jars containing 3.8 liters of solution, with no headspace. Silverside minnow were arbitrarily added, two at a time, until each replicate contained 10 fish, within approximately one hour of initial WAF solution collection. The test exposure chambers were held in a water bath maintained at 20 ± 1 °C for the first day of the study, then were moved to an incubator for the remainder of the study, at the same temperature. The chambers were sealed with teflon lined screw caps to minimize volatilization. Exposure concentrations with surviving fish were renewed at each 24-hour interval during conduct of the study by siphoning the final solutions out of each test chamber, leaving only enough volume so that the organisms were not distressed. A sample of each final solution was retained for water quality analysis. At least one composite 40 ml final sample of each concentration (20 ml for each replicate) was also taken at the renewal for chemical analysis. The newly prepared solution was then carefully siphoned into each test chamber.

The fish were exposed to a control and five nominal concentrations (3 ppm, 12 ppm, 22 ppm, 52 ppm, and 97 ppm) of Whole Light Alkylate Product. The control consisted of the same dilution water, test conditions, and test organisms with no added test material. The fish in each test chamber were observed daily for mortality at 1, 3, 6, and 24 hour intervals. Daily observations at 1, 3, and 6 hours were made with the jar lids remaining on, to prevent volatilization. The 24, 48, 72 and 96 hour observations were made with the lids removed, during renewal or at termination. Abnormalities such as surfacing, coughing, loss of equilibrium and discoloration were documented, if observed, at each observation period. The criterion for death was a lack of opercular movement. Fish remaining alive at the end of the study were killed by an anesthetic overdose of approximately 200 ppm Finquel® solution, placed in labeled plastic bags, and frozen prior to measurement.

Test Procedure-Water Quality:

Water quality parameters of dissolved oxygen (D.O.), pH, salinity, and temperature were measured at study initiation and daily in a portion of the freshly-prepared initial sample. These water quality parameters were also measured daily in final replicate samples. Water

quality was performed only on final samples from test chambers that contained some living organisms at the previous observation period, and in initial samples from chambers with some living organisms present. Dissolved oxygen was measured with a YSI Model 57 D.O. Meter with a Model 5739 D.O. probe. The pH was measured with an Orion Model 520A Digital pH/mV Meter with an Orion Model 81-02 Combination pH Electrode. Salinity was measured with a Spartan Model A366ATC Salinity Hand Refractometer. Temperature was measured with a hand-held thermometer, with a stainless steel thermocouple.

Test Procedure-Chemical:

Chemical analysis was performed on single 40 ml samples, both initial and final, of the control and all exposure concentrations at 0, 24, 48, 72, and 96 hours after test initiation. Chemical analysis was performed only on final samples from test chambers that contained some living organisms at the previous observation period, and in initial samples from chambers with some living organisms present. The samples were collected in 40 ml vials with no head space, and transferred to the Analytical Chemistry group for analysis. The concentration of Whole Light Alkylate Product (measured concentration) in each sample was determined by using purge-and-trap and a gas chromatograph equipped with a flame ionization detector (GC-FID) following the methods developed in the methods validation study (Appendix 2, Study No. 65969) Details of the method are included in the appendix. The following components of Whole Light Alkylate Product were quantified: 2,3-dimethyl butane, 2,4-dimethyl pentane, 2,2,4-trimethyl pentane, 2,5-dimethyl hexane, 2,3,4-trimethyl pentane, 2,3,3-trimethyl pentane, and 1-methyl-1-ethyl-cyclopentane. Based on the method validation study, these components represent 68% of the composition of Whole Light Alkylate Product. All chemical analysis (summary in the Analytical Chemistry Report) was performed by C.W. Chuang of the Analytical Chemistry Group.

Statistical Analysis:

Daily LC_{50} values were calculated on the basis of mortality data and nominal/measured dose levels. Statistical analysis of the data was calculated by a computer software LC_{50} program developed by Stephan et al. (7). This program statistically calculates the LC_{50} using binomial probability analysis, moving average angle analysis, and probit analysis. The LC_{50} was also calculated using the Spearman-Karber method (8,9). These different methods of analyzing the data are used since no one method of analysis is appropriate for all possible sets of data that may be obtained (10). The method selected for analysis of the data present in this report was determined by the characteristics of the data base.

Daily measured dose levels, for each concentration, were a cumulative total of all sample values evaluated between the 0 hour initial sample and the final sample, inclusive, for that time period. Measured dose levels were the cumulative total of all measured test material components, for each concentration. In cases where the measured component levels were below that component's detection limit, a zero value was included in the addition of components. The detection limits used were determined in the methods validation study. For the 96 hour time period (all samples), a standard deviation was also calculated. The average measured levels for each time period were used along with corresponding survival data to produce measured LC_{50} and NOEC values. Also for each concentration, all initial sample values were averaged, and all final sample values were averaged. The percent difference between initial and final averages was used to calculate the average percent retention at each exposure period.

Data Storage:

The study was conducted according to the EPA Good Laboratory Practice Standards (40 CFR Part 792) (11). Raw data (Appendix 3) and the original final report are maintained in the Archives of Stonybrook Laboratories Inc. located in Pennington, New Jersey.

RESULTS:

The LC_{50} values for the 96-hour static-renewal toxicity study of Whole Light Alkylate Product to silverside minnow (*Menidia beryllina*) are summarized in Table 3. Based on nominal exposure concentrations, the 24 and 48 hour LC_{50} values were both 34 ppm, while the 72 and 96 hours values were 32 ppm and 27 ppm, respectively. Based on daily measured exposure concentrations, the 24, 48, 72, and 96 hour LC_{50} values were 564 ppb, 525 ppb, 483 ppb, and 426 ppb, respectively. All LC_{50} values were determined by binomial probability analysis. Cumulative mortality data for this study are presented in Table 4. Behavioral observations for this study are presented in Table 5.

Water quality parameters of pH, dissolved oxygen, salinity, and temperature were performed only on initial samples from chambers with some living organisms present, and on final samples from test chambers that contained some living organisms at the previous observation period. Mean values and the range/standard deviation for each test chamber are summarized in Tables 6 and 7.

The measured concentrations of Whole Light Alkylate Product in the test chambers were determined by purge-and-trap/gas chromatography (Appendix 1). The concentrations listed in this appendix are based on the coding system identified in the raw data where the first character represents the test concentration group as listed in the protocol; the second character represents either an initial (I) or a final (F) sample; and the third and fourth characters represent the hour of the sampling period. The measured exposure concentrations and calculated averages of the samples collected during the study and the percent retention for average initial and final samples collected during the study are summarized in Table 8 and 9. The chemical analysis techniques used in this study were developed during the Methods Validation Study (Study 65969). A copy of this study is provided in Appendix 2.

DISCUSSION:

The temperature and salinity monitored during the study remained within acceptable limits. The pH values remained consistent among concentrations and dissolved oxygen levels remained above 60% saturation in all doses. No mortality or behavioral abnormalities were observed in the control chambers throughout the study. Total mortality was observed in the two highest concentrations, 52 ppm and 97 ppm, by 6 hours. At test termination, no mortality was observed in the 3 ppm and 12 ppm concentrations, with partial mortality observed in the 22 ppm concentration (6 fish, 30%). The 96-hour LC_{50} for Whole Light Alkylate Product to silverside minnow under static-renewal test conditions was, therefore, 27 ppm based on nominal exposure concentrations, and 423 ppb based on average measured exposure concentrations.

Samples of the control and exposure concentrations were collected daily and quantitatively analyzed using purge-and-trap/gas chromatography (GC). The concentrations were quantitated by GC using standard Whole Light Alkylate Product component standards. Test material retention from the static-renewal procedure ranged from 57.1 to 90.1%. Daily initial measured concentrations indicated consistent exposure of the test fish to Whole Light Alkylate Product throughout the study. A trace amount of test material (1 ppb) was quantified in the final control samples at 48 hours.

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TABLE 1: Characteristics of MTC Well Water (2 Year Average)

Parameter Measured	Concentration
Dissolved Oxygen	5.2 ppm
pH	7.52
Conductivity	444 µmhos
Total Hardness ($CaCO_3$)	197 mg/L
Alkalinity ($CaCO_3$)	143 mg/L
TSS	<5 mg/L
Ammonia (Distillation as N)	<1 mg/L
Phosphorus (Total as P)	<0.06 mg/L
Sulfate	60 mg/L
COD	<7 mg/L
Cyanide	<0.005 mg/L
Antimony	<0.04 mg/L
Arsenic	<0.01 mg/L
Barium	0.14 mg/L
Beryllium	<0.003 mg/L
Cadmium	<0.001 mg/L
Chromium	<0.002 mg/L
Copper	0.09 mg/L
Iron	<0.1 mg/L
Lead	<0.002 mg/L
Magnesium	18.3 mg/L
Manganese	<0.01 mg/L
Mercury	<0.0002 mg/L
Nickel	<0.05 mg/L
Fluoride	0.1 mg/L
Selenium	<0.004 mg/L
Silver	<0.002 mg/L
Zinc	<0.05 mg/L
TOC	<1 mg/L
NO_3-N	<2 mg/L
Thallium	<0.1 mg/L
Phenols	<0.005 mg/L
Lindane	<0.01 µg/L
Methoxychlor	<0.05 µg/L
Endrin	<0.01 µg/L
Toxaphene	<4 µg/L

TABLE 2: Length and Weight Measurements Taken During the Acute Toxicity Study of Whole Light Alkylate Product to Silverside Minnow

Test Conc.	Rep.	Standard Length (mm) X	s	Weight(g) X*	s**	LF***
Pretest Sample		13	3	0.02	0.02	0.06
Control	A	14	2	0.02	0.02	0.06
Control	B	15	2	0.03	0.01	0.09
3 ppm****	A	13	2	0.02	0.01	0.05
3 ppm	B	13	1	0.02	0.01	0.06
12 ppm	A	14	2	0.02	0.01	0.06
12 ppm	B	15	2	0.03	0.01	0.08
22 ppm	A	14	1	0.03	0.01	0.07
22 ppm	B	15	2	0.03	0.02	0.08
52 ppm	A	14	2	0.03	0.01	0.07
52 ppm	B	14	2	0.03	0.02	0.07
97 ppm	A	13	2	0.02	0.01	0.06
97 ppm	B	14	2	0.03	0.01	0.07

* X = Mean Value

** s = Standard Deviation

*** Loading Factor: g/Liter = $\frac{\text{Average Weight (g/fish) x No. fish in Test Chamber}}{\text{Test Chamber Vol. (Liters)}}$

**** Two fish were not measured (lost during retrieval)

TABLE 3: Acute Toxicity of Whole Light Alkylate Product to Silverside Minnow

	LC_{50}* (95% Confidence Limits)**			
	24 Hrs	48 Hrs	72 Hrs	96 Hrs
Nominal	34 ppm (22-52 ppm)	34 ppm (22-52 ppm)	32 ppm (22-52 ppm)	27 ppm (12-52 ppm)
Measured	561 ppb (242-1,300 ppb)	522 ppb (210-1,300 ppb)	482 ppb (225-1,300 ppb)	423 ppb (160-1,300 ppb)

* All LC_{50} values calculated using Binomial Probability Analysis.
** The 95% confidence limits presented above are not actually confidence limits because the LC_{50}s were determined by binomial probability. The limits are statistically sound conservative bounds that are above 95% for the sample size used in this study.

	NOEC**			
	24 Hrs	48 Hrs	72 Hrs	96 Hrs
Nominal	22 ppm	22 ppm	22 ppm	12 ppm
Measured	242 ppb	210 ppb	225 ppb	160 ppb

** All NOEC values calculated using Fisher's exact test.

TABLE 4: Cumulative Mortality During the Acute Toxicity Study of Whole Light Alkylate Product to Silverside Minnow

Exposure Time	Nominal Concentration (ppm)					
	Control	3	12	22	52	97
Day 0:						
1 hrs.	0/20	0/20	0/20	0/20	6/20	10/20
3 hrs.	0/20	0/20	0/20	0/20	15/20	18/20
6 hrs.	0/20	0/20	0/20	0/20	20/20	20/20
24 hrs.	0/20	0/20	0/20	0/20	20/20	20/20
Day 1:						
1 hrs.	0/20	0/20	0/20	0/20	20/20	20/20
3 hrs.	0/20	0/20	0/20	0/20	20/20	20/20
6 hrs.	0/20	0/20	0/20	0/20	20/20	20/20
24 hrs.	0/20	0/20	0/20	0/20	20/20	20/20
Day 2:						
1 hrs.	0/20	0/20	0/20	0/20	20/20	20/20
3 hrs.	0/20	0/20	0/20	0/20	20/20	20/20
6 hrs.	0/20	0/20	0/20	0/20	20/20	20/20
24 hrs.	0/20	0/20	0/20	1/20	20/20	20/20
Day 3:						
1 hrs.	0/20	0/20	0/20	1/20	20/20	20/20
3 hrs.	0/20	0/20	0/20	1/20	20/20	20/20
6 hrs.	0/20	0/20	0/20	1/20	20/20	20/20
24 hrs.	0/20	0/20	0/20	6/20	20/20	20/20

TABLE 5: Behavior Observations During the Acute Toxicity Study of Whole Light Alkylate Product To Silverside Minnow

Behavior of Survivors	Nominal Concentration (ppm)					
Exposure Time	Control	3	12	22	52	97
Day 0:						
1 hrs.	20A	20A	20A	20A	13A,1BJ	10A
3 hrs.	20A	20A	20A	20A	5B	2B
6 hrs.	20A	20A	20A	20A	---	---
24 hrs.	20A	20A	20A	20A	---	---
Day 1:						
1 hrs.	20A	20A	20A	20A	---	---
3 hrs.	20A	20A	20A	20A	---	---
6 hrs.	20A	20A	20A	20A	---	---
24 hrs.	20A	20A	20A	19A,1W	---	---
Day 2:						
1 hrs.	20A	20A	20A	19A,1W	---	---
3 hrs.	20A	20A	20A	19A,1W	---	---
6 hrs.	20A	20A	20A	19A,1W	---	---
24 hrs.	20A	20A	20A	19A	---	---
Day 3:						
1 hrs.	20A	20A	20A	19A	---	---
3 hrs.	20A	20A	20A	19A	---	---
6 hrs.	20A	20A	20A	19A	---	---
24 hrs.	20A	20A	20A	14A	---	---

A - Normal
B - Quiescent
J - Ceased Swimming
W - Slower Respiration

TABLE 6: Summary of Initial Water Quality Measurements Taken During the Acute Toxicity Study of Whole Light Alkylate Product to Silverside Minnow

Test Concentration	Temperature (°C) X*	Range	pH Range
Control	20.4	19.9-20.9	8.14-8.29
3 ppm	20.2	19.7-20.8	8.14-8.34
12 ppm	20.3	19.6-20.9	8.14-8.35
22 ppm	20.3	19.6-20.7	8.15-8.31
52 ppm	19.6	**	8.25**
97 ppm	19.6	**	8.22**

Test Concentration	D.O. (ppm) X*	Range	Salinity (ppt) X*	Range
Control	7.2	7.0-7.2	20.8	20.0-21.0
3 ppm	7.1	7.0-7.4	20.8	20.0-21.0
12 ppm	7.0	6.9-7.2	21.0	***
22 ppm	7.1	7.0-7.3	20.5	20.0-21.0
52 ppm	7.0	**	21.0	**
97 ppm	7.0	**	21.0	**

* X = Mean Value
** Parameter only measured once during the study due to total mortality by 24 hours.
*** Parameter remained the same throughout the study.

TABLE 7: Summary of Final Water Quality Measurements Taken During the Acute Toxicity Study of Whole Light Alkylate Product to Silverside Minnow

Test Conc.	Rep.	Temperature (°C) X*	Temperature (°C) Range	pH Range
Control	A	20.1	19.7-20.6	8.21-8.29
Control	B	20.0	19.5-20.8	8.22-8.30
3 ppm	A	20.0	19.4-20.7	8.23-8.32
3 ppm	B	20.0	19.4-20.7	8.24-8.32
12 ppm	A	20.0	19.4-20.6	8.23-8.31
12 ppm	B	20.1	19.4-20.6	8.22-8.30
22 ppm	A	20.2	19.6-20.9	8.23-8.34
22 ppm	B	20.3	19.6-20.8	8.23-8.33
52 ppm	A	19.6	**	8.25**
52 ppm	B	19.7	**	8.26**
97 ppm	A	19.8	**	8.23**
97 ppm	B	19.9	**	8.22**

Test Conc.	Rep.	D.O. (ppm) X*	D.O. (ppm) Range	Salinity (ppt) X*	Salinity (ppt) Range
Control	A	6.4	6.0-6.8	21	20-21
Control	B	6.3	6.2-6.4	21	20-21
3 ppm	A	6.3	6.1-6.6	21	20-21
3 ppm	B	6.4	6.3-6.4	21	***
12 ppm	A	6.2	5.8-6.6	21	***
12 ppm	B	6.2	6.0-6.4	21	21-22
22 ppm	A	6.0	5.8-6.3	21	***
22 ppm	B	6.1	6.0-6.2	21	20-21
52 ppm	A	6.4	**	22	**
52 ppm	B	6.3	**	21	**
97 ppm	A	6.2	**	21	**
97 ppm	B	6.3	**	22	**

* X = Mean Value
*** Parameter only measured once during the study due to total mortality by 24 hours.
**** Parameter remained the same throughout the study.

TABLE 8: Measured Exposure Concentrations During the Acute Toxicity Study of Whole Light Alkylate Product to Silverside Minnow

All values in ppm

Sample	0 hr. Initial	24 hr. Final	24 hr. Initial	48 hr. Final	48 hr. Initial	72 hr. Final	72 hr. Initial	96 hr. Final
Control	ND	ND	ND	0.001	ND	ND	ND	ND
3 ppm	0.027	0.027	0.025	0.037	0.046	0.032	0.077	0.049
12 ppm	0.375	0.111	0.116	0.112	0.054	0.054	0.267	0.188
22 ppm	0.196	0.197	0.189	0.260	0.310	0.198	0.649	0.450
52 ppm	1.370	1.234	*	*	*	*	*	*
97 ppm	1.432	1.168	*	*	*	*	*	*

* Intial or final samples not taken due to complete mortality at 24 hours.

TABLE 9a: Daily Cumulative Averages of the Measured Exposure Concentrations Determined for the Acute Toxicity Study of Whole Light Alkylate Product to Silverside Minnow

All values in ppm

Sample	24 hr. Avg.	48 hr. Avg.	72 hr. Avg.	96 hr (All Samples) Avg.	Std. Dev.
Control	ND	ND	ND	ND	ND
3 ppm	0.027	0.029	0.032	0.040	0.017
12 ppm	0.242	0.178	0.137	0.160	0.112
22 ppm	0.196	0.210	0.225	0.306	0.165
52 ppm *	1.302	1.302	1.302	1.302	0.096
97 ppm *	1.300	1.300	1.300	1.300	0.186

TABLE 9b: Initial/Final Averages and Percent Retention of the Measured Exposure Concentrations Determined for the Acute Toxicity Study of Whole Light Alkylate Product to Silverside Minnow

All values in ppm

Sample	Average of Initial Samples	Average of Final Samples	% Retention
Control	ND	ND	NC
3 ppm	0.044	0.036	82.9
12 ppm	0.203	0.116	57.1
22 ppm	0.336	0.276	82.2
52 ppm *	1.370	1.234	90.1
97 ppm *	1.431	1.168	81.6

* Only one intial and one final sample taken due to complete mortality at 24 hours.

APPENDIX 1

STONYBROOK LABORATORIES INC.

To: J. F. Barbieri

Date: May 9, 1995

From: C.W. Chuang

CC: M.T. Benkinney

RE: ANALYSIS OF WHOLE LIGHT ALKYLATE PRODUCT IN WATER ACCOMMODATED FRACTION (WAF)

STUDY NO: 65911

The analysis of whole light alkylate product in WAF was performed following a purge-and-trap/gas chromatography procedure recently validated in-house (Study no. 65969). The results are revised as follows:

Table 1.1 Concentration of analytes in stock solutions prepared at 0 hour

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1I00	0	ND*	ND	ND	ND	ND	ND	ND	----
2I00	3	0.004	ND	0.009	ND	0.005	0.009	ND	0.027
3I00	12	0.189	0.029	0.074	ND	0.030	0.053	ND	0.375
4I00	22	0.073	0.016	0.046	ND	0.020	0.039	0.002	0.196
5I00	52	0.499	0.080	0.279	0.047	0.168	0.294	0.003	1.370
6I00	97	0.263	0.067	0.326	0.069	0.256	0.447	0.004	1.432

* ND = not detected at the method detection limit (ref: Study no. 65969).

Table 1.2 Concentration of analytes of 24-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F24	0	ND	ND	ND	ND	ND	ND	ND	----
2F24	3	0.004	ND	0.008	ND	0.005	0.010	ND	0.027
3F24	12	0.033	0.008	0.028	ND	0.015	0.027	ND	0.111
4F24	22	0.083	0.016	0.042	ND	0.019	0.037	ND	0.197
5F24	52	0.633	0.088	0.210	0.022	0.096	0.183	0.002	1.234
6F24	97	0.529	0.088	0.225	0.027	0.104	0.190	0.005	1.168

Table 2.1 Concentration of analytes in stock solutions prepared at 24 hours

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1I24	0	ND	ND	ND	ND	ND	ND	ND	----
2I24	3	0.007	ND	0.006	ND	0.004	0.008	ND	0.025
3I24	12	0.035	0.009	0.029	ND	0.015	0.028	ND	0.116
4I24	22	0.066	0.013	0.046	ND	0.022	0.042	ND	0.189

Table 2.2 Concentration of analytes of 48-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F48	0	ND	ND	ND	ND	ND	ND	0.001	0.001
2F48	3	0.009	ND	0.010	ND	0.006	0.012	ND	0.037
3F48	12	0.036	0.008	0.027	ND	0.014	0.027	ND	0.112
4F48	22	0.098	0.015	0.060	ND	0.028	0.057	0.002	0.260

Table 3.1 Concentration of analytes in stock solutions prepared at 48 hours

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1I48	0	ND	ND	ND	ND	ND	ND	ND	----
2I48	3	0.016	ND	0.010	ND	0.006	0.013	0.001	0.046
3I48	12	0.014	0.004	0.012	ND	0.008	0.016	ND	0.054
4I48	22	0.112	0.023	0.067	0.009	0.033	0.061	0.005	0.310

Table 3.2 Concentration of analytes of 72-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F72	0	ND	ND	ND	ND	ND	ND	ND	----
2F72	3	0.011	ND	0.007	ND	0.004	0.010	ND	0.032
3F72	12	0.016	0.004	0.012	ND	0.007	0.015	ND	0.054
4F72	22	0.075	0.012	0.043	ND	0.021	0.043	0.004	0.198

Table 4.1 Concentration of analytes in stock solutions prepared at 72 hours

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1I72	0	ND	ND	ND	ND	ND	ND	ND	----
2I72	3	0.021	0.004	0.020	ND	0.011	0.021	ND	0.077
3I72	12	0.090	0.017	0.061	ND	0.030	0.061	0.008	0.267
4I72	22	0.308	0.045	0.119	0.009	0.057	0.111	ND	0.649

Table 4.2 Concentration of analytes of 96-hour WAF from test containers

Sample ID	Prepared concentration (ppm)	2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total (ppm)
1F96	0	ND	ND	ND	ND	ND	ND	ND	----
2F96	3	0.014	ND	0.012	ND	0.007	0.016	ND	0.049
3F96	12	0.066	0.008	0.045	ND	0.022	0.047	ND	0.188
4F96	22	0.216	0.027	0.087	ND	0.040	0.080	ND	0.450

Please call me to discuss the results.

APPENDIX 2

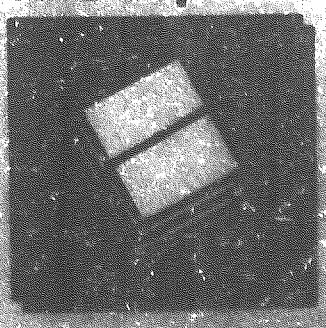
Stonybrook
Laboratories Inc.

Methods Validation for the Analysis of Whole Light Alkylate Product in Water Accommodated Fraction (WAF) Using Purge-and-Trap and GC/FID

Stonybrook Laboratories Inc.
Princeton, NJ

Study Number: 65969

Final Report



STONYBROOK LABORATORIES INC.

REPORT RELEASE

LIAISON: C.A. SCHREINER

STUDY NUMBER: 65969

CRU NUMBER: 94194

TEST ARTICLE: WHOLE LIGHT ALKYLATE PRODUCT

STUDY TITLE: METHODS VALIDATION FOR THE ANALYSIS OF WHOLE LIGHT ALKYLATE PRODUCT IN WATER ACCOMMODATED FRACTION (WAF) USING PURGE-AND-TRAP AND GC/FID

RESULTS:

The development and validation of a purge-and-trap/gas chromatography (PT/GC) method for the analysis of water acclimated fractions (WAF) of whole light alkylate product and the subsequent determination of optimal WAF equilibration times has been completed. The method was developed and validated using seven C6-C8 alkane and cycloalkane standards which represent 68% of the whole light alkylate product. The sensitivity and precision of the assay were validated at the 5 part-per-billion (PPB) level for each of the seven component standards in water. Using this technique, it was determined that the whole light alkylate product freshwater WAF reached equilibrium in approximately 24 hours at a total WAF concentration (sum of n=7 components) of 1.6 parts-per-million (PPM). The saltwater WAF reached equilibrium in approximately 12 hours at a total concentration (sum of n=7 components) of 0.9 PPM.

T.A. Roy 11/30/95
T.A. Roy Date
Study Director

C.A. Schreiner 11/30/95
C.A. Schreiner Date
Vice-President

C.R. Mackerer 2/1/95
C.R. Mackerer
President Date

DISTRIBUTION:
All above, Liaison/C.A. Schreiner, Archives
STUDY NO. 65969

STATEMENT OF COMPLIANCE

The undersigned hereby state that Study No. 65969, Methods Validation for the Analysis of Whole Light Alkylate Product in Water Accommodatated Fraction (WAF) Using Perge-and-Trap and GC/FID, was conducted in compliance with the Good Laboratory Practice Regulations as published in 40 CFR Part 792 Federal Registrar Volume 54-158, 8/17/89 in all aspects with the following exceptions:

The strength, purity and composition or other characteristics to define the test substance was not determined by the testing facility. The methods of synthesis, fabrication, or derivation of the test substance are the responsibility of the sponsor and the data are located at the sponsor's facility.

The purity of purchased reference materials was not determined by the testing facility. It is not known if the purity determination of these chemicals by the supplier were performed under GLPs.

The data acquisition or analysis software on the HP MS DOS operating system used in the study has not been validated in-house.

No bulk inventory usage log was mantained for the test chemicals or analytical standards.

T. A. Roy
Study Director

G. A. Ratusina
Study Sponsor

TABLE OF CONTENTS

SUMMARY

The development and validation of a purge-and-trap/gas chromatography (PT/GC) method for the analysis of water acclimated fractions (WAF) of whole light alkylate product and the subsequent determination of optimal WAF equilibration times has been completed. The method was developed and validated using seven C6-C8 alkane and cycloalkane standards which represent 68% of the whole light alkylate product. The sensitivity and precision of the assay were validated at the 5 part-per-billion (PPB) level for each of the seven component standards in water. Using this technique, it was determined that the whole light alkylate product freshwater WAF reached equilibrium in approximately 24 hours at a total WAF concentration (sum of n=7 components) of 1.6 parts-per-million (PPM). The saltwater WAF reached equilibrium in approximately 12 hours at a total concentration (sum of n=7 components) of 0.9 PPM.

EXPERIMENTAL

EXPERIMENTAL DESIGN SUMMARY:

Seven C6-C8 alkanes and cycloalkane, which represent 68% of whole light alkylate product, were selected as the monitored analytes for the in-house method validation. The analyte in methanol solution was spiked into 5 mL deionized water. The aqueous solution were loaded into the purge-and-trap sparger by a Luer Lock syringe. The analytes were then purged out by helium from the aqueous phase to the vapor phase at ambient temperature. The vapor was transferred and consequently trapped in a sorbent tube. After the purge was completed, the sorbent tube was then backflushed and heated. The analytes were swept by helium onto the head of the GC column where the separation and detection took place. The evaluations included measuring each compound's response sensitivity, reproducibility, and purge efficiency. Once the analytical procedure had been verified, a WAF of Whole Light Alkylate Product was generated and evaluated at different time intervals to demonstrate the suitability of the proposed WAF generation procedure.

TEST SUBSTANCES:

ANALYTE NAME	CRU #	LOT #	EXPIRATION	PURITY
2-methylbutane (isopentane)	94570	03859DG	9/99	99%
2,3-dimethylbutane	94565	LA-44304	9/99	99%
2,4-dimethylpentane	94565	LA-44304	9/99	99%
2,5-dimethylhexane	94565	LA-44304	9/99	99%
2,2,4-trimethylpentane	94565	LA-44304	9/99	99%
2,3,4-trimethylpentane	94565	LA-44304	9/99	99%
hexane (surrogate)	110-54-3*	42H06471	1/99	99%
2,3,3-trimethylpentane	94591	244X-5S	10/99	99%
1-methyl-ethylcyclopentane	94590	2360	10/99	99%

*CAS Number

Chemical purity and stability data for reference standards purchased commercially were provided by the suppliers (Supelco, Sigma, Wiley, API Standard Reference Materials). The data provided by the suppliers is archived with the raw data.

APPARATUS AND REAGENTS:

Syringe--5 mL gas-tight glass with Luer Lock.
Micro syringes--10 µL, 25 µL, 50 µL, 100 µL, and 250 µL.
GC vials--Glass with Teflon-lined screw caps.
Volumetric flasks--Variable volume size with ground-glass stoppers.
Analytical balance--0.0001 g.
Methanol--HPLC grade.

Secondary working standard mixes--Two standard mixes of the eight whole light alkylate component alkanes plus hexane were prepared by mixing their individual stock standard in methanol for a concentration of 100 µg/mL: mix I: isopentane, 2,3, 3-trimethypentane, and 1-methyl-1-ethylcyclopentane and mix II contained the remaining 5 analytes plus the surrogate, hexane.

Calibration standards--Five levels of standards (approximately 1, 5, 10, 25 and 50 µg/mL) were prepared from the secondary working standard mixes.

Spiking surrogate standard--An approximately 10 µg/mL of hexane was prepared in methanol from the stock standard. This solution was spiked in all blanks, spikes, and samples prior to analysis.

Storage and handling precautions --All solutions (except stock standards) were stored at 4°C and labeled with study number, names, concentrations, and expiration date. All solutions will be disposed of upon release of the final report

PROCEDURE:

Set up the acquisition sequence on the Waters chromatography data system.

A 5 mL Luer Lock syringe is filled to overflowing with deionized water which has also been heated to boiling to remove residual volatile organics. The plunger is replaced and the water compressed to the 5 mL mark. The plunger is pulled back slightly to allow for the addition of 5 µL of calibration standard or spiking surrogate standard. After the solution is loaded to the P&T, press START on the LSC 2000 front panel to start the purge-and-trap procedure.

Initial calibration - Run five levels of calibration standards following the procedure described above and calculate the response factor (RF) of the individual analytes based on equation (I):

$$RF = A_S/C_S \qquad (I)$$

where:

A_S: peak area count of analyte

C_S: amount in nanograms (e.g., 5 µL of a 1.0 µg/mL solution = 5 ng) of the calibration standard injected into the syringe

Calculate the average response factor (RF_{ave}) and standard deviation (SD) of five-level calibration standards. Calculate the relative standard deviation (%RSD = (SD/RF_{ave}) x 100) of the calibration using Microsoft Excel (version 4.0). If %RSD is < 20%, then the RF_{ave} of the analytes is used for quantitation. If %RSD > 20%, the first degree linear regression (forced through zero) with r> 0.99 is used for quantitation (re: quantitative analysis section).

Sample analysis - The analysis follows the steps described above. Samples were analyzed only once using one of two duplicate sample vials except when a need for further confirmation arose or when dilutions were required to bring the response of the analytes within the range of the calibration standards. The duplicate sampling vials were used in these cases.

WAF GENERATION AND EVALUATION:

Two types of WAFs of Whole Light Alkylate Product were evaluated to demonstrate equilibrium and maintenance of test material. A WAF prepared with freshwater was evaluated at 0,1,3,6,24,36,48,60 and 72 hours after preparation while a WAF prepared with saltwater was evaluated at 0,1,3,6,12,24,36 and 48 hours after preparation. The WAFs were generated following modification of the procedure used by Anderson, et al (1974, Marine Biol., 27: 75-88). Two WAFs were prepared, using each water type, containing 50 ppm of Whole Light Alkylate Product. One WAF of each water type was prepared in a bottle filled to the neck to minimize headspace ("XXX1X" sample designation, e.g, sample "3FW2B" is a 3-hour, freshwater, type 1 WAF, the second of duplicate samples collected), while the second WAF of each water type was prepared in a bottle filled to the shoulder to maximize product-water contact ("XXX2X" sample designation). Duplicate samples were collected from each bottle (except for time zero "XXX2X" series) at the specified time periods, with one sample analyzed using the methodology determined from the in-house validation and the other sample acting as a backup. All samples were collected in 40 ml glass vials with no headspace. The concentration in each flask was quantified to evaluate the consistency of the WAF with time, water type and stirring procedure.

GOOD LABORATORY PRACTICES:

This study was conducted according to the EPA Good Laboratory Practice Standards outlined in 40 CFR Part 160, Federal Register Vol. 54, No.158, 8/17/89.

Test Substance(s) Characterization - The methods of synthesis, fabrication, and/or derivation of the test materials is the responsibility of the sponsor. In addition, the stability, identity, strength, purity and composition of other characteristics which identify the test materials are the responsibility of the sponsor. The test article data are located at the sponsor's facility.

Chemical purity and stability data for reference and control standards purchased commercially, with the exception of 2,3,3-trimethylpentane and 1-methyl-ethylcyclopentane, were provided by the suppliers (Supelco, Sigma). The latter two compounds were assayed for purity at Stonybrook Laboratories Inc. These data and those provided by the suppliers are archived with the raw data.

RECORDS MAINTAINED:

The study file contains but is not limited to the following records or verified copies of:

Notice of Intent to Initiate Study
Request for Testing
Sponsor Protocol Amendment Approval Memo
Study Protocol and Amendments
Technical Personnel Records
Reagents and Equipment Inventory
Chemical Repository Unit (CRU) Dispensing Records
Study Notebook Records

RESULTS & DISCUSSION

METHOD EVALUATION/VALIDATION:

The use of the PT/GC technique for the analysis of whole light alkylate product WAF was based on a review of the test article composition and the anticipated composition of the WAF. The use of PT/GC runs throughout the EPA analytical methods series for drinking water (500), municipal/industrial effluent water (600) and wastewater (8000). The method has been tentatively validated for the analysis of gasoline range organics (GRO) in the last year and drafts of the method were made available by the Office of Solid Waste (OSW) prior to the expected promulgation in late 1994.

Six alkanes and one cycloalkane were selected (representing 68% of the components of the test material) for the in-house evaluation/validation. Hexane was chosen as the surrogate. The EPA procedure for the evaluation of method performance is an appropriate standard by which to assess in-house method validation. Determination of the method detection limit (MDL), limit of detection (LOD) and limit of quantitation (LOQ) provide an excellent measure of the sensitivity and precision of the procedure. MDL is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the value is above zero. The LOD is the lowest concentration that can be determined statistically differently from the blank. LOD is numerically defined as three times the standard deviation from replicate measurements of standard. LOQ is the level above which quantitative results may be obtained and is numerically defined as ten times the standard deviation from replicate measurements of standards. The LOD, LOQ and MDL were determined from replicate measurements of the analytes and surrogate in water at 5 PPB. In general, the per component MDL was slightly below 5 PPB. The LOD, LOQ and MDL for each of the compounds is reported in table I.

WAF GENERATION AND EVALUATION:

Two types of WAF were generated to evaluate the affect of mixing and headspace on final WAF concentration. The concentration of test article components was significantly higher (factor of 2) in the "minimal headspace" type WAF as compared to the "maximum phase interface" type WAF. Table II reports the time course of WAF concentration for the individual and summed seven analytes monitored for both freshwater (through 72 hours) and saltwater (through 48 hours). The surrogate recoveries, which were essentially quantitatve, are also reported for each WAF sample analyzed.

WAF concentration of test material peaked at approximately 12 hours in saltwater (0.9 PPM) and 24 hours in freshwater (1.6 PPM) using the "minimal headspace"WAF generation procedure. This can be seen more clearly in Figure 1 where the "Total" column data in table II for freshwater and saltwater WAF concentrations are plotted vs time of sampling in a histogram format. Figure 2 plots the individual component concentrations for freshwater and saltwater WAF vs sampling time and shows that the relative concentration of the individual test article WAF components is largely maintained over the mixing period. Figures 3 and 4 compare the 24 hour WAF concentration of the test article components with the actual concentration of the components in the test article. These experimentally observed results can be predicted with a reasonable degree of accuracy if the water solubility or octanol/water partition coefficients of the components are taken into consideration.

Table 1

Summary Sheet for LOD, LOQ and MDL Determinations for Whole Light Alkylate Product WAF Components and Surrogate

Peak#	Compound	Rt. (min.)	Area count						
			Run1	Run2	Run3	Run4	Run5	Run6	Run7
1	2,3-dimethylbutane	8.065	37422	31896	36712	34842	30650	20817	46749
2	hexane (surr)	8.850	35696	30159	34788	34045	28001	19625	46398
3	2,4-dimethylpentane	9.600	41098	34678	40270	37909	33107	22357	52058
4	2,2,4-trimethylpentane	11.420	43358	36274	41736	41538	36182	25682	54862
5	2,5-dimethylhexane	12.750	40754	34303	39308	40101	34222	24153	51361
6	2,3,4-trimethylpentane	13.480	41512	35834	41050	42265	37296	25760	53336
7	2,3,3-trimethylpentane	13.680	41454	36507	41116	42461	38096	35948	41716
8	1-methyl-1-ethylcyclopentane	15.115	46856	42044	46061	49500	44715	45595	47700

Peak#	Compound	Rt. (min.)	PFSCL ODLOQ R1	PFSCL ODLO QR2	PFSCL ODLO QR3	PFSCL ODLOQ R4	PFSCL ODLOQ R5	PFSCL ODLO QR6	PFSCL ODLO QR7	RF(ave)	Std. Dev.	%RSD	Std. Dev. (ppb)	LOD (ppb)	LOQ (ppb)	t value*	MDL (ppb) =Std. Dev. x t
			Response factor=Area count/S (ng)														
1	2,3-dimethylbutane	8.065	7484.4	6379.2	7342.4	6968.4	6130.0	4163.4	9349.8	6831.1	1573.9	23.0	1.2	3.5	12	3.71	4.3
2	hexane (surr)	8.850	7139.2	6031.8	6957.6	6809.0	5600.2	3925.0	9279.6	6534.6	1637.6	25.1	1.3	3.8	13	3.71	4.6
3	2,4-dimethylpentane	9.600	8219.6	6935.6	8054.0	7581.8	6621.4	4471.4	10411.6	7470.8	1805.8	24.2	1.2	3.6	12	3.71	4.5
4	2,2,4-trimethylpentane	11.420	8711.8	7254.8	8347.2	8307.6	7236.4	5136.4	10972.4	7995.2	1774.6	22.2	1.1	3.3	11	3.71	4.1
5	2,5-dimethylhexane	12.750	8150.8	6860.6	7861.6	8020.2	6844.4	4830.6	10272.2	7548.6	1656.2	21.9	1.1	3.3	11	3.71	4.1
6	2,3,4-trimethylpentane	13.480	8302.4	7166.8	8210.0	8453.9	7459.2	5152.0	10667.2	7915.8	1658.5	21.0	1.0	3.1	10	3.71	3.9
7	2,3,3-trimethylpentane	13.680	8290.8	7301.4	8223.2	8492.2	7619.2	7189.6	8343.2	7922.8	538.9	6.80	0.34	1.0	3.4	3.71	1.3
8	1-methyl-1-ethylcyclopentane	15.115	9371.2	8408.8	9212.2	9900.0	8943.0	9119.0	9540.0	9213.5	471.2	5.11	0.26	0.77	2.6	3.71	0.95

* t value at 99% confidence interval

Table II
Continued

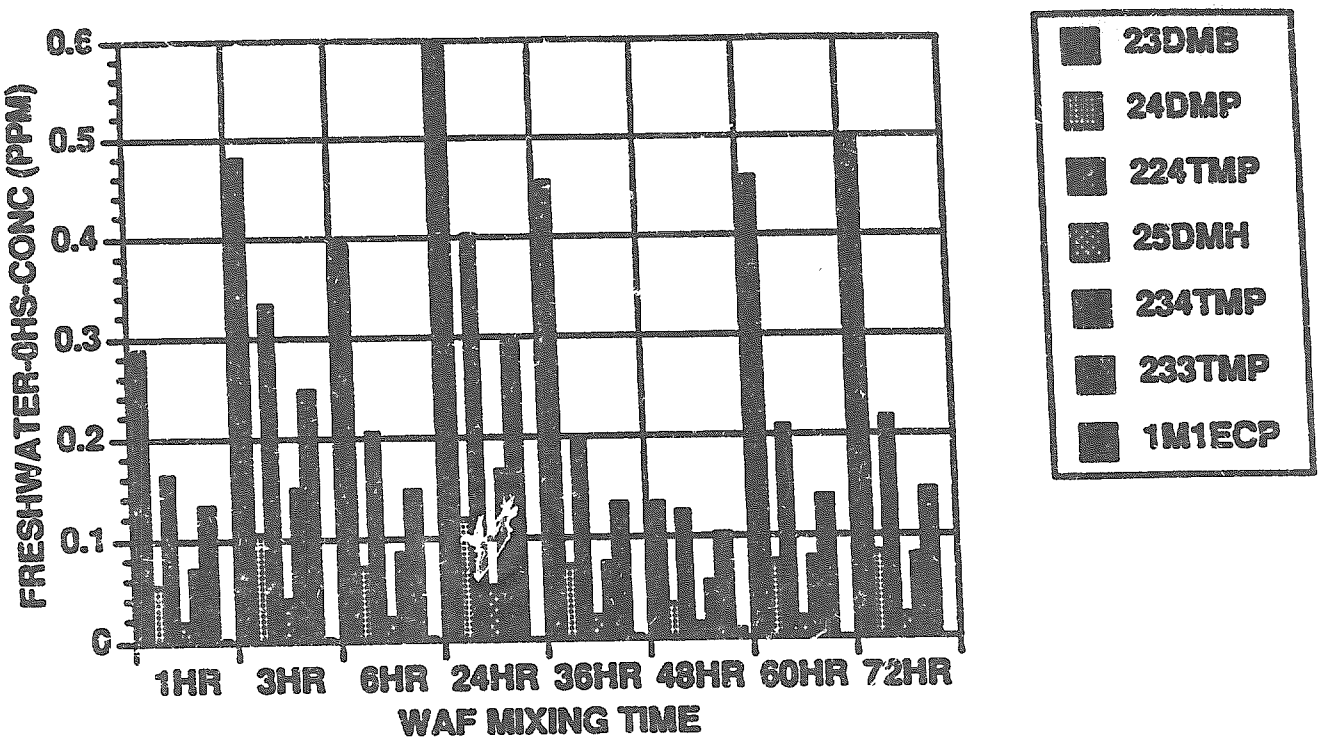
		2,3-dimethyl butane	2,4-dimethyl pentane	2,2,4-trimethyl pentane	2,5-dimethyl hexane	2,3,4-trimethyl pentane	2,3,3-trimethyl pentane	1-methyl-1-ethyl-cyclopentane	Total	hexane (surr) recovery (%)
	RF(ave)	*7524.2*	*8025.4*	*8569.9*	*8495.5*	*8756.4*	*8818.6*	*9204.0*		*7378.6*
Data file	DF*									
36FW1A	20	0.455	0.072	0.197	0.022	0.073	0.133	0.002	0.953	111
36SW1A	10	0.372	0.056	0.145	0.016	0.055	0.101	0.002	0.747	114
36FW2A	20	0.206	0.041	0.128	0.014	0.055	0.103	0.000	0.547	109
36SW2A	10	0.174	0.036	0.117	0.014	0.052	0.100	0.000	0.492	102
48FW1A	20	0.132	0.031	0.125	0.016	0.054	0.102	0.006	0.465	110
48SW1A	10	0.327	0.049	0.134	0.013	0.050	0.099	0.000	0.672	109
48FW2A	20	0.331	0.062	0.186	0.019	0.074	0.140	0.000	0.812	81
48SW2A	10	0.166	0.033	0.100	0.012	0.044	0.084	0.000	0.438	114
60FW1A	20	0.456	0.074	0.206	0.021	0.076	0.138	0.000	0.972	102
60FW2A	20	0.242	0.055	0.184	0.024	0.082	0.146	0.000	0.733	109
72FW1A	20	0.500	0.081	0.216	0.023	0.079	0.143	0.000	1.042	109
72FW2A	20	0.222	0.046	0.142	0.016	0.059	0.110	0.000	0.595	101

*dilution factor

Data file format - e.g., 36FW1A = 36 hour collection time, freshwater, type "1" WAF (see experimental section), "A", first of two (duplicate) samples collected at the indicated time point.

Figure 2

Individual Monitored Component Concentrations in Whole Light Alkylate Product Freshwater and Saltwater WAFs over 48-72 Hours



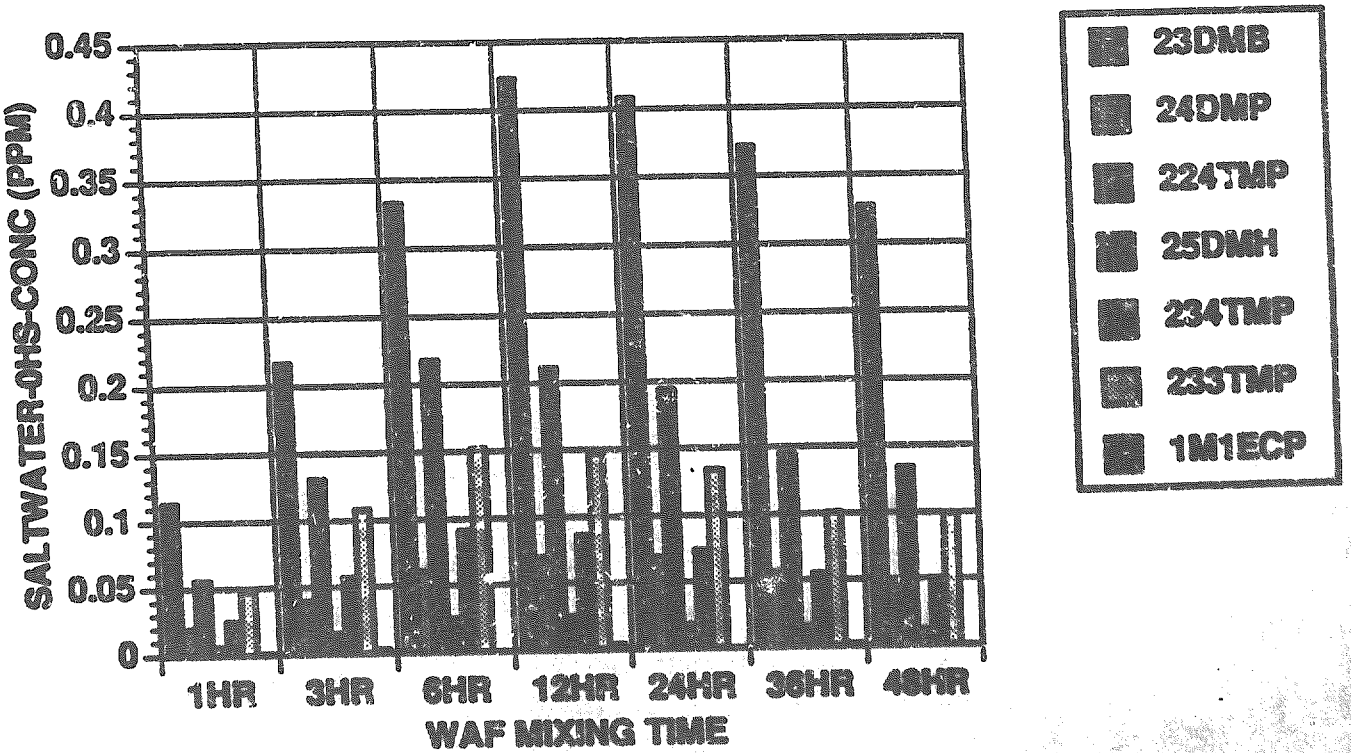


Figure 4

Comparison of Whole Light Alkylate Product Alkane Concentrations In The Neat Material With Their 24 Hour WAF Concentration (Saltwater)

65969 10/26/94 DATA